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AMILTON B. G. ROBINSON

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EDITOR'S VIEWPOINT

Geyer (p. 590) fed vanadum (V_2O_5) to hamsters on cariogenic diets and injected it into animals on the same diets. In a limited number of animals he noted remarkable reduction of new enamel caries and cessation of progress of dentin caries. This interesting effect of a trace element appears to deserve wider study on significant groups of animals.

Helman and Mitchell (p. 596), noting discrepancies between oral lactobacillus counts and caries in hamsters, postulated the idea that coprophagy might affect the oral lactobacillus count. A lack of correlation between salivary lactobacillus counts and caries in three groups of hamsters on different diets and a positive correlation between oral lactobacillus counts and coliform counts tend to make questionable the significance of salivary lactobacillus counts in the hamster or other coprophagic animals. The simplified hamster caries scoring method used will be of interest.

Kantorowicz and Birman (p. 601) have investigated the relationship of air contact to acid production in saliva. They found that, among other factors, acid production in saliva is dependent upon oxygen tension in the mixture. This is most important in investigations of salivary acid production. The authors suggest that it also may be of importance in vivo, for example in mouth breathers.

Chilton and Fertig (p. 606) discuss the use of comparisons of portions in estimation of sample size. This material should be of great value in planning experimental procedures. Adequate planning to obtain statistically acceptable material is of prime importance to the investigator.

The problem of bacteremias following tooth extraction in relationship to systemic disease is of importance in general health. Kraus, Casey, and Johnson (p. 613) classified the non-hemolytic streptococci recovered in the blood after 160 extractions. Twenty-nine per cent of these streptococci were of Lancefield groups A to O, 47.5 per cent of the viridans group were *Streptococcus mitis*, while only one strain resembled *S. thermophilus*, two *S. equinis*, five *S. sanguis*, and five *S. salivarius*. On the other hand, *S. mitis* has been found in 23 per cent of subacute bacterial endocarditis and *S. sanguis* in 44 per cent. It appears that few of the organisms found in bacteremias of dental origin are potential producers of subacute bacterial endocarditis. This biologic fact probably protected the vast majority of those individuals who develop post-extraction bacteremias without benefit of preoperative antibiotic or chemotherapy.

Levy and Gorlin (p. 622) found changes in the temporomandibular joints of scorbutic guinea pigs compatible with those described in other bones of scorbutic animals. The findings are illustrated by photomicrographs.

Hutton (p. 626) describes the application of the Hotchkiss-MacManus periodic acid-leucofuchsin method for determining the purity of enamel separated by the Manly-Hodge method or by other methods.

The Proceedings of the March, 1953 General Meeting of the International Association for Dental Research are published in this issue. The amount of information in capsule form which is afforded by the 212 abstracts is amazing. Any student of dentistry—investigator, educator, clinician—can gain much useful information from each page of these Proceedings, and by the same token each reader of the JOURNAL who does not carefully peruse the Proceedings will miss an unusual opportunity.

The British Section's Proceedings are a welcome addition to the JOURNAL. The seventeen papers on various investigations will afford those outside of England an opportunity to become familiar with the dental investigators in England.

The American Dental Association has adopted a resolution to direct attention of the public to inaccurate dentifrice claims, to urge manufacturers and advertising agents to cease "practices which tend to destroy the confidence of the public in all advertising," and to urge dentists to communicate scientific information to their patients. Much of the material on which dentifrice claims are based has been published in this JOURNAL. An example of the use of that material by advertisers appears in this issue. Does the public or the general dental profession know that the published material showed only reduction in acid production on tooth surfaces, not reduction in dental caries? Can the average individual read carefully enough to know that "all day decay acid immunity" is not decay immunity?

H. B. G. R.

VANADIUM, A CARIES-INHIBITING TRACE ELEMENT IN THE SYRIAN HAMSTER

CARLOS F. GEYER, FREIE UNIVERSITÄT, BERLIN, GERMANY

THE limits of the caries-inhibiting effect of fluorine have been demonstrated by the experiments carried out on a large scale in the United States and by the theoretical work of Hodge.[1] Hodge has compiled the results of the investigations on the chemical composition of the hard tooth substances and of bone in Table I.[2]

Fluorine is always found in ponderable amounts in the hard tissues and therefore must be considered as a normal constituent of these tissues. On the contrary, the elements filed in the last line, whose presence in demonstrable amounts could be established only by spectroscopic methods, should be called real trace elements of the hard tissues. We asked ourselves whether there is an element among these trace elements whose chemical, biochemical, and catalytic properties might be able to increase the resistance of the tooth to caries if it were present in sufficient quantities in the dentin and enamel. A thorough study of these elements resulted in the conclusion that vanadium might be able to increase the resistance to caries.

Vanadium, an element of the fifth subgroup of the periodic system, has an entirely metallic character and is used as an addition to steel and cast iron in amounts of 0.1 to 0.2 per cent to make them harder, tougher, more ductile, and more resistant to blows. This is partly due to the great deoxidizing ability of vanadium. Vanadium compounds are qualified as oxygen transferrers in catalytic oxidation processes, as in the preparation of sulfuric acid by the contact process, of phthalic anhydride by oxidation of naphthalene, and in the preparation of perborates. The atom of vanadium can give off up to five electrons, but it cannot collect any. The vanadium ion with five valences measures about 0.4 Å in semidiameter; the one with four valences has a radius of 0.61 Å. The research work covering the apatites and the substitution of their individual elements by other elements within the apatite lattice[3-5] revealed that a part of the phosphorus ions of the hydroxyl apatite can be replaced by carbon, arsenic, or vanadium,[4] or by arsenic, vanadium, sulfur, silicon (C?), and aluminum.[5] McConnell[4] established the fact that only about 10 per cent of the phosphorus ions can be replaced by other ions, an interesting analogy to the limited substitution of hydroxyl ions by fluorine ions in mottled teeth, where only about 4 per cent of the theoretically possible substitution takes place.[6] From a purely crystallographic view, the substitution of phosphorus ions by vanadium ions is quite possible, because the vanadium ion has a radius only slightly larger than the phosphorous ion, the latter measuring 0.32 Å. However, the isolated inorganic components of the hard tissues cannot be considered

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TABLE I

CHEMICAL CONTENTS OF ENAMEL, DENTIN, CEMENTUM, AND BONE

	ENAMEL	DENTIN	CEMENTUM COMPACT BONE
Water	2.3	13.2	32
Organic matter	1.7	17.5	22
Ash	96.0	69.3	46
In 100 g. of Ash:			
Calcium	36.1	35.3	35.5
Phosphorus	17.3	17.1	17.1
Carbon dioxide	3.0	4.0	4.4
Magnesium	0.5	1.2	0.9
Sodium	0.2	0.2	1.1
Potassium	0.3	0.07	0.1
Chloride	0.3	0.03	0.1
Fluorine	0.016	0.017	0.015
Sulfur	0.1	0.2	0.6
Copper	0.01		
Silicon	0.003		0.04
Iron	0.0025		0.09
Zinc	0.016	0.018	
	Whole teeth		Bone
Lead	0.0071 to 0.037		0.002 to 0.02
Small amounts of:	Ce, La, Pr, Ne, Ag, Sr, Ba, Cr, Sn, Mn, Ti, Ni, V, Al, B, Cu, Li, Se,		

alone. Analysis of the hard tooth substances by the electron microscope has shown that the organic and inorganic constituents of the hard tissues are not strictly separated from each other, as it would appear under the ordinary microscope. Perdok[7] found a remarkable correspondence of the periods of the apatite lattice with the periods of the fibrillar proteins of the organic framework. This fundamentally supports the opinion of Eisenberger,[8] that within the hard tissues "there is a continuous series of solid solutions, where only one factor remains constant, i.e., the apatite lattice." Therefore, when discussing the possibilities of substitution in this lattice, we also must consider the complex proteins of the organic matter. In nature, vanadium has the ability to act as a central atom of such proteins. *Phallusia mamillata* and other ascidians (family of the tunicates) contain in their blood the so-called mulberry cells, a chromogen dye, which does not combine with oxygen in a dissociable form like hemoglobin or hemocyanin. Henze, Stöhr, and Müller[9] assume that this dye probably acts like chlorophyll in a system of reduction by which a reduction of carbon dioxide and a synthesis of carbohydrates is performed. The striking accumulation of vanadium in these animals is also interesting. Their ash contains up to 15 per cent vanadium, although only slight traces of vanadium can be found in 200 liters of evaporated sea water. Therefore, it might theoretically be possible that vanadium ions, embedded in enamel and dentin, could increase the hardness of the hydroxyl apatite as well as the cohesion between the organic and inorganic matter.

When discussing the theoretical possibilities of vanadium acting as a caries-inhibiting trace element, Dr. Eggers-Lura drew my attention to the research work of Rygh,[10-14] who has studied the action of trace elements on the develop-

ment of the entire organism. He came to the well-defined conclusion that the mineralization of the hard tissues was promoted markedly by the strontium and vanadium during the period of development of the animals. Zinc, thallium, and barium, on the contrary, had a restraining effect on the mineralization of these tissues. When strontium and vanadium were missing in the synthetic diet he gave his animals, mineralization was extremely bad. However, if the three others were not present, either separately or altogether, the mineralization was remarkably good. Rygh also found the largest number of carious teeth at post-mortem examination of the vanadium and strontium deficient animals. In an additional series of tests, the animals were given increased amounts of these trace elements. Rygh stated, "Strontium and vanadium caused a mineralization of marked degree and mostly when given both at the same time. Barium, zinc and thallium were all effecting decalcification, mostly marked when all three were given in overdosage at the same time. Carious teeth were here only seen on overdosage of barium, zinc and thallium at the same time, to a lesser degree on overdosage of thallium alone."[10] In the presence of increased amounts of vanadium and strontium in the synthetic diet employed in these tests, no carious teeth appeared. The results of these experiments strengthened our hypothesis that vanadium might more or less prevent the effect of a caries-producing diet in the test animals.

EXPERIMENTAL

Ten animals from two litters of the same age of *Cricetus auratus* were taken over when about 30 days old. The animals were kept in glass boxes to avoid ingestion of other metallic trace elements, with wood shavings for bedding. Diets and distilled water were available ad libitum. Two control animals received a normal diet, while eight animals were given the Keyes diet. After twenty-four days, the first symptoms of fissure caries appeared on the molars of all eight experimental animals. The Keyes diet was continued with four animals, while the other group of four animals were given a daily addition of 0.08 mg. of vanadium pentoxide to the caries-producing diet. We chose this compound because it contains, besides vanadium, only oxygen, and because this compound acts as a catalyzator. Twenty-five days later the animals were sacrificed. The animals of the first group with a pure caries-producing diet showed severe carious lesions and destructions on the molars, whereas on the animals of the second group (Keyes diet + 0.08 mg. V_2O_5 daily) the caries of the enamel had not advanced into the dentin (Figs. 1 to 3).

Another group of ten hamsters was treated under the same experimental conditions. After twenty-one days of the Keyes diet, half of the animals were given a daily addition of only 0.04 mg. V_2O_5. The result was the same: very large carious lesions in the first group and a cessation of caries of the enamel in the second group (Fig. 4).

In order to get an exact control of the amount of V_2O_5 effective, each animal in a third series received a subcutaneous injection of 0.07 mg. V_2O_5 once a week under the skin of the neck. In order to avoid the action of other trace elements,

the vanadium salt was dissolved in bidistilled water. The injections were endured quite well, although the solution was not isotonic. They were started at the same time that the feeding with the caries-producing diet started. Six weeks later, the typical lesions of several teeth and severe fissure caries were observed on the check animals, which were given the pure caries-producing diet, while on the animals receiving vanadium only isolated cases of fissure caries of the enamel were observed. This beginning enamel caries remained stationary until the animals were sacrificed (Fig. 5).

Fig. 1. Fig. 2.

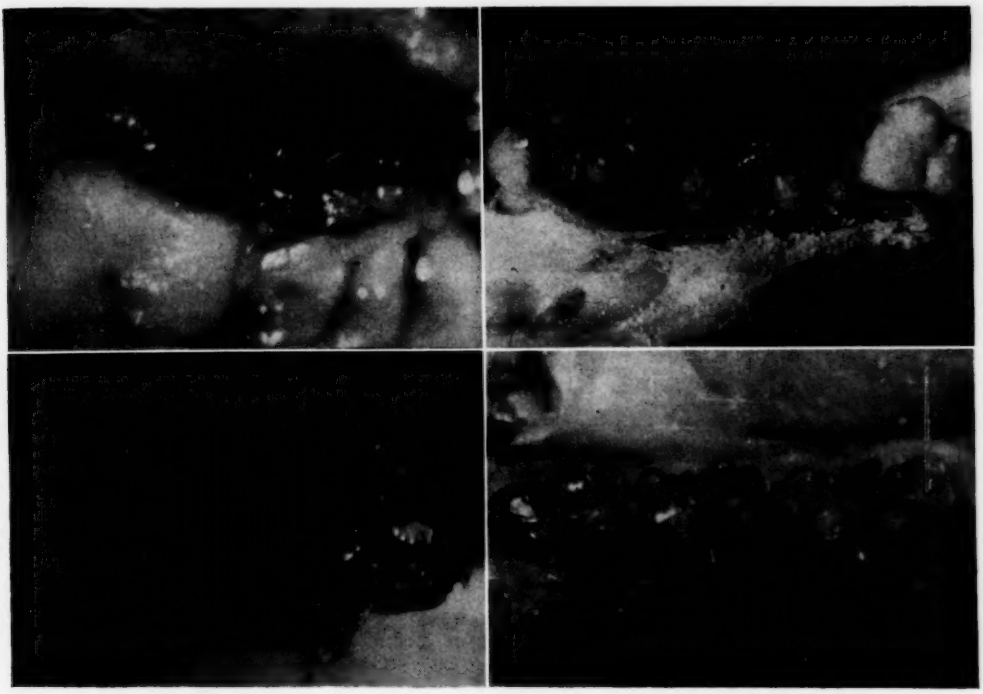


Fig. 3. Fig. 4.

Fig. 1.—Upper right jaw of Syrian hamster. Keyes diet for seven weks. Last molar completely destroyed, first molar carious.
Fig. 2.—Lower left jaw of Syrian hamster, macerated. Keyes diet for seven weeks. Crown of second molar completely destroyed, first molar carious.
Fig. 3.—Upper jaw of Syrian hamster. Keyes diet for twenty-four days, then Keyes diet + 0.08 mg. V_2O_5 daily for twenty-five days. Incipient caries of enamel became obvious after twenty-four days. No progression of caries under influence of vanadium pentoxide.
Fig. 4.—Upper jaw of Syrian hamster. Keyes diet + 0.04 mg. V_2O_5 daily. Identical caries-inhibiting effect.

These series of tests were repeated several times. They show that vanadium pentoxide undoubtedly has an inhibiting effect on the dentinal caries in the Syrian hamster. Furthermore, no new caries of the enamel arose as long as the vanadium salt was given. Fortuitously, a characteristic change of color in the dentin of the molars of the sacrificed hamsters receiving vanadium was obtained. This resembles the reddish brown color of the vanadium pentoxide

Fig. 5.

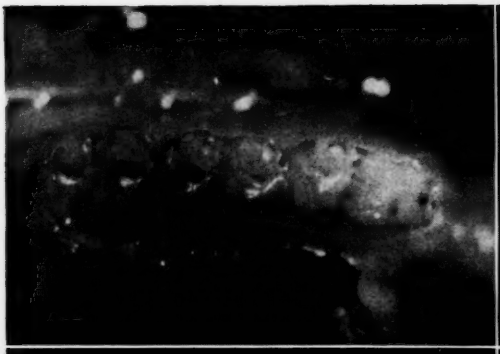


Fig. 6.

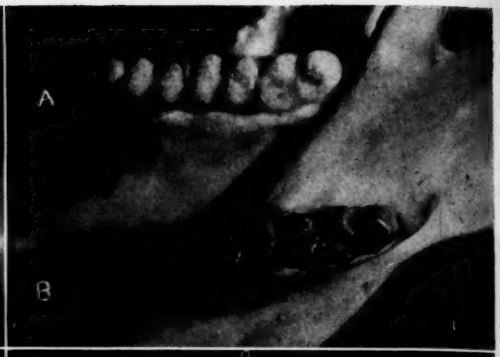


Fig. 7.

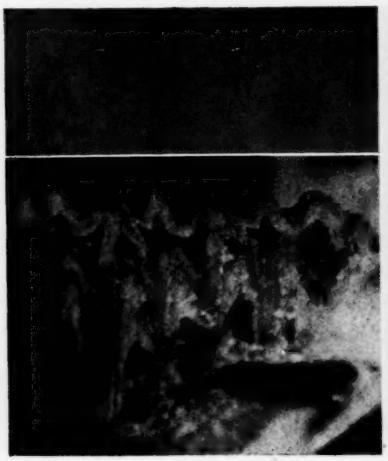
Fig. 8.

Fig. 5.—Upper jaw of Syrian hamster. Keyes diet for six weeks and during the same time a subcutaneous injection of 0.07 mg. V_2O_5 in bidistilled water once a week. Incipient caries of enamel without progression.

Fig. 6.—Two halves of lower jaws of Syrian hamsters receiving Keyes diet + 0.08 mg. V_2O_5 daily. Jaw *A* was prepared by stripping off soft tissues and boiling for a long time in tube water. Jaw *B* was put in anthill for four weeks after the animal was sacrificed and then boiled in tube water. While boiling, the reddish brown color appeared, which resembles the color of V_2O_5. This staining did not appear on the teeth of the control animals.

Fig. 7.—Lower jaw of a hamster receiving vanadium, ground section. Third molar lost through grinding. In dentin of two molars dark ribbon of vanadium is visible. Base of continuously erupting incisor shows same staining of newly formed dentin.

Fig. 8.—Molar of a hamster receiving vanadium, ground section. Caries in a fissure of enamel. Undermining tendency of enamel caries in *a*. The caries has not penetrated into the dentin. In *b* and *c* ribbons of vanadium in dentin.



A.

B.

C.

Fig. 9.—Lower jaw of a hamster receiving vanadium, ground section. *A*, Every fissure of the three molars shows beginning carious lesion in enamel. *B*, Left molar in higher magnification. Vanadium ribbons in dentin. *C*, Left fissure of first molar in still higher magnification. The typical fissure caries of the enamel has not penetrated into the dentin. Below the fissure, the vanadium ribbon covers whole thickness of dentin.

(Fig. 6). When the animals had not received vanadium together with the caries-producing diet, the molars did not change their color when treated identically. Ground sections of these stained teeth were observed. The staining was present in the dentin in more or less broad ribbons (Fig. 7). The caries of the enamel did not penetrate into the dentin, the latter being stained close below the dentinoenamel junction (Figs. 8 and 9). An analogous staining of the enamel could not be observed.

DISCUSSION

From a pharmacologic viewpoint, vanadium has some interesting properties. According to Lendle,[15] the usual vanadium compounds have no specific protein-precipitating properties. The same author stated that pharmacologically vanadium stands near phosphorus and particularly close to arsenic, but that it also has some properties related to mercury and lead. Arsenic and lead are deposited in the bones and teeth in an extraordinarily high degree when an increased ingestion of their salts into the organism occurs. Carow[16] and Hintze[17] proved that lead can also be deposited in fully erupted teeth. Howe[18] demonstrated the deposition of lead in teeth of individuals poisoned by lead and in animal experiments. He found a deposit of lead especially in the interglobular dentin and in the dentinal tubuli. It seemed as if the lead had penetrated into certain zones of the dentin. Maulbetsch and Rütishauser[19] showed, by the spectroscopic method, that the whole amount of lead contained in the teeth was found in the dentin and especially in the dentin of the roots. A similar pharmacodynamic behavior of the vanadium is quite possible.

In further experiments we are trying to obtain litters of Syrian hamsters which have been influenced by vanadium in utero. The aim is to investigate whether the enamel of the young animals has a higher caries resistance through intrauterine adsorption of vanadium ions.

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LACTOBACILLI AND DENTAL CARIES IN THE HAMSTER

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AN ASSOCIATION between oral *Lactobacillus acidophilus* and dental caries in the human being has been demonstrated by several investigators,[1-7] while a few workers[8-10] have been unable to show such an association. This relationship also has been studied in rodents by Rosebury,[11] Harrison,[12] McClure and Hewitt,[13] Wakeman, Smith, Zepplin, Sarles, and Phillips,[14] Stephan, Fitzgerald, McClure, Harris, and Jordan,[15] and Orland,[16] with rather inconclusive results.

The purpose of this investigation was to determine what, if any, relationship exists between *Lactobacillus acidophilus* (La) counts and caries in the hamster, and at the same time to determine if a relationship exists between La counts from the mouth of the hamster and from fecal contamination. This last point was deemed to be of importance in view of the fact that several investigators have found no difference between the strains of oral and intestinal lactobacilli,[16-20] and since hamsters indulge in coprophagy it was conceivable that through contamination with fecal La, the La counts from the mouths of the hamsters could be greatly affected.

EXPERIMENTAL

Two main groups of hamsters were used in this study. The first group consisted of forty female hamsters maintained on a laboratory chow diet until they were 3 months old. At that time they were placed on the following experimental diets for four months: Group A—whole wheat flour 30 per cent, whole powdered milk 30 per cent, cornstarch 20 per cent, alfalfa meal 4 per cent, sodium chloride 1 per cent; Group B—same as Group A, with 15 per cent confectioners' sugar added; Group C—same as Group A, plus water containing 20 ppm of NaF.

After these hamsters were on their respective diets for about five weeks, they were mated and their offspring constituted the animals of the second group of this study. At 21 days of age these offspring were weaned and all put on Diet A. At 30 days of age they were all put on Diet B for a period of 100 days. In all, only twenty-five offspring survived the entire experiment. The greatest loss occurred prior to weaning, and may have been due to the inadequacy of the diet which caused the mothers to eat many of the young.

At the end of the respective experimental periods the mothers and the offspring were sacrificed, the jaws preserved in 80 per cent alcohol, stripped of the flesh, and scored for caries by a simplified method using a net reticule, American Optical No. 422 (21.15 mm. diameter, 5 mm. square ruled into ½ mm.

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squares), in the eyepiece of a dissecting microscope. The grid could be focused directly above the teeth and the degree of caries involvement determined by counting the number of squares over a carious area. A score of 352 would denote complete coronal destruction of all the molars. In order to determine the accuracy of this method, thirty jaws that had been scored by a method described in a previous article[21] were rescored by the grid method and a very good correlation was found (Fig. 1).

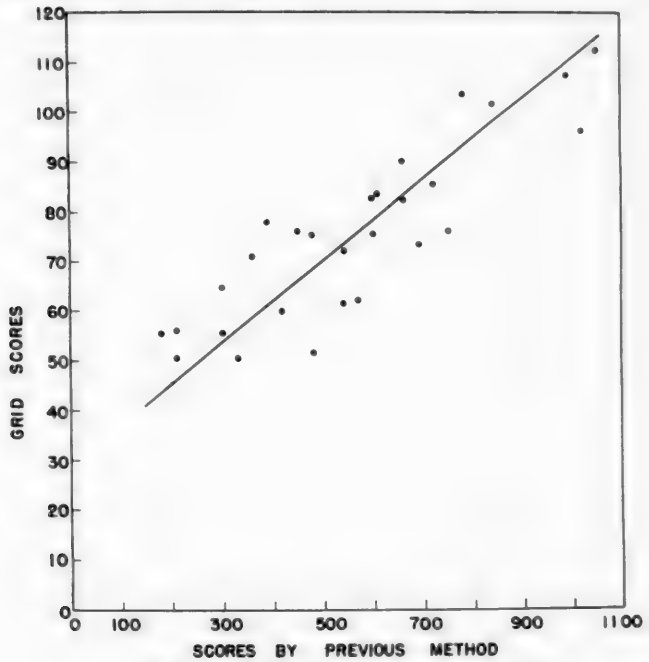

Fig. 1.—Comparison of two scoring methods.

Lactobacillus and coliform counts on the mothers were made prior to sacrifice, while on the young they were made both one month prior to sacrifice and at the time of sacrifice. To determine the number of lactobacilli and coliform bacteria present in the mouths of the hamsters, the following procedures were used.

Method of Collecting Saliva.—The unanesthetized hamster is firmly held in the left hand. The right hand is used to hold a white blood cell pipette connected to a small suction apparatus.[22] The blood pipette is inserted in the hamster's mouth, worked around the whole mouth, including the teeth, and simultaneously the screw on the top of the suction apparatus is opened to create a negative pressure. Saliva and matter around the teeth are slowly aspirated into the bulb of the blood pipette. When sufficient material is collected, the contents of the blood pipette are emptied into a micro test tube of 0.25 ml. capacity.[23] This procedure is repeated until 50 to 100 microliters (.05 to .1 ml.) are collected. Micro pipettes of the Linderstrøm-Lang type[24] were used to quantitate the sample and make the necessary dilutions.

Lactobacilli and Coliform Counts.—Dilutions of 1:10, 1:100, and 1:1,000 in triplicate were made from each sample of saliva. One hundred fifteen microliters of each dilution were added to test tubes containing 25 c.c. of SL agar,[25] stirred and plated out. After incubation at 37° C. the number of La colonies per plate were counted. To determine the number of coliform organisms in the saliva, 115 microliters of each dilution were added to eosinmethylene blue agar and plated out. After incubation the number of coliform colonies was counted. The final counts were expressed as the number of colonies per cubic millimeter of saliva. To determine the accuracy of this micro method for determining La in the saliva, human saliva was run by both the macro and micro methods. The counts obtained by the micro method were in close agreement with those obtained by the macro method.

RESULTS

Table I summarizes the data obtained from the mother hamsters. No significant difference (at the 0.01 level) was found between the mean occlusal scores, La counts, and coliform counts of the three groups. Since the mothers were not given the caries-producing diets until they were 3 months old, the degree of caries involvement was of a very low order as expected, since adult hamsters are rather refractory to caries.

TABLE I

DATA FOR MOTHER HAMSTERS

GROUP	NUMBER	DIET	OCCLUSAL SCORES MEAN ± STANDARD ERROR	LA COUNTS (NUMBER OF COLONIES PER CUBIC MILLIMETER OF SALIVA)	COLIFORM COUNTS (NUMBER OF COLONIES PER CUBIC MILLIMETER)
I	14	A	17.0 ± 2.7	179 ± 32.5	417 ± 91.7
II	13	A + sugar	22.5 ± 3.7	360 ± 162.0	593 ± 292.0
III	13	A + F^-	28.5 ± 5.2	348 ± 103.0	343 ± 174.0

Since it was desirable to see if any relationship existed between the caries scores and lactobacillus counts for each individual hamster regardless of group, correlation curves were plotted and correlation coefficients were calculated. No apparent correlation was found between the occlusal scores and lactobacillus counts, either by plot or by correlation coefficient, the r value being –0.12. The correlation curve relating lactobacillus counts and coliform counts showed a positive correlation, and the correlation coefficient was 0.769, indicating a significant ($P = 0.0001$) degree of association.

The caries scores of the offspring were higher than the mothers, ranging from 31.5 ± 13.8 in a female group to 84.0 ± 8.5 in one of the male groups. Although the "p" values for the differences between the mean caries scores of the three groups were not significant, there appears to be a decrease in the incidence of caries in both the males and females fed fluoride prenatally. Further studies using greater numbers of animals are under way to test this point.

In the offspring, a significant degree of correlation was obtained between the individual occlusal scores and the La counts taken one month prior to

sacrifice ($r = 0.67$, $P = 0.007$), but not between the occlusal scores and the La counts taken at the time of sacrifice. The correlation between the lactobacilli and coliform counts was positive, but not to the significant extent found in the case of the mothers.

DISCUSSION

Since in human beings the lactobacillus counts are indicative of current caries activity and thus may indicate caries experience in the future, it was thought to be worth while to see if the same holds true for hamsters. If such a relationship had been found in hamsters, a useful tool would have been found by which it would be possible to determine the influence of various factors on caries incidence before the end of the usual experimental period. No such correlation (to a significant extent) could be demonstrated in adult hamsters fed three different kinds of diets. There is reason to believe that through contamination by fecal La, the true oral La count may be obscured, since a significant correlation was obtained between lactobacillus counts and coliform counts in the saliva of these hamsters.

In view of the fact that a significant correlation was obtained in the offspring between the occlusal scores and lactobacillus counts taken one month prior to sacrifice (but not between caries and La counts at the time of sacrifice, in either the offspring or mothers) it is a temptation to postulate a precaries or early caries association of lactobacilli with dental caries in the hamster. However, because of the factor of contamination by fecal La, the significance of the findings is open to question.

It would seem that unless coprophagy can be prevented for a considerable period prior to the taking of a sample of saliva, the lactobacillus counts probably cannot be used as an index of caries activity in the hamster.

SUMMARY

1. No correlation was found to exist between caries experience and lactobacillus counts of adult hamsters maintained on three different diets. A significant degree of association was found between the lactobacillus counts and coliform counts; therefore, it appears that intestinal lactobacilli introduced into the mouth of the hamster via the feces may affect the true lactobacillus count.

2. A limited number of offspring of the females used in this experiment experienced the prenatal influence of the previously described diets until 21 days of age and then were fed a high sugar diet for 100 days. A high degree of correlation was obtained between the occlusal scores and lactobacillus counts taken one month prior to sacrifice, but not between the occlusal scores and lactobacillus counts taken at the time of sacrifice. Because of the demonstrated factor of contamination by fecal lactobacilli, the significance of these lactobacillus counts is questionable.

3. A micro method for the determination of lactobacilli and other organisms in hamster saliva is presented, as well as a simplified method of scoring hamster caries.

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ACID PRODUCTION IN SALIVA IN RELATION TO AIR CONTACT

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IN THE abundant literature on acid production in saliva no reference is made to its dependence upon air contact, that is to the salivary pO_2.

In one individual, salivary pH has been shown to be variable with the diet, with the time since the last meal, and even with the season.[3] During sleep, the pH range seems to be lower.[6] A number of other factors, psychic included, influence salivary composition, so that it seems necessary to repeat experiments with one single subject for some weeks in order to arrive at reliable figures.

Individual differences in the production of acids in kept saliva appear to be large, dependent upon the degree of glycolysis and the oral neutralization time. Fosdick[5] classified people into four groups, namely, those with (1) rapid production of acids and rapid neutralization, (2) rapid production of acids and slow neutralization, (3) slow production of acids and rapid neutralization, and (4) slow production of acids and slow neutralization. According to Fosdick, Group 2 shows higher and Group 3 lower caries activity. The widely accepted relation between salivary acidification and dental caries calls for interest in the measurement of acid production.

We wish to draw attention to the influence of the extent of the air-saliva interface on the production of acid in experiments in vitro. We encountered this problem in experiments concerned with the influence of dentifrices on salivary acid production. Following the method of Bibby, Goldberg, and Chen[1] evaluating the decalcification potential, we tried to obtain the acid-producing saliva under more or less physiologic circumstances, e.g., by rinsing with a solution of glucose. Originally, acid production was measured by titration, according to Bibby's method, four and twenty-four hours after collection, but later after only one hour's incubation at 37° C. Stagnation of saliva during four hours or more will not be found under natural conditions, at least not during daytime, and for this reason, and to prevent putrefaction and fungus growth, the one-hour period was chosen.

In preliminary experiments it was observed that duplicate tests did not agree if the air bubble content of salivary samples was markedly different, the foam-containing specimen always producing less acid. This was interpreted* as a possible pO_2 influence, and systematic experiments, conducted under aerobic or anaerobic conditions, confirmed this supposition.

PROCEDURES

A 5 per cent solution of "Dextropur" (pure d [+] glucose) is adjusted to pH 6.6 electrometrically. At this pH carbonic acid has no influence on the

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*Our thanks are due Dr. R. Brinkman, professor of biochemistry in the University of Groningen (Holland), for this suggestion.

titration. In the first series 0.2 per cent NaCl and 0.02 per cent Na_2HPO_4 were added previously. As acid production was not different without this addition, it later was omitted.

Sterilization of the glucose solution, necessary for storage and for constant initial condition, is attained by heating it twice to 100° C. Treated in this way, the solution remains unchanged for four weeks.

Seven and one-half cubic centimeters of the glucose solution is equally distributed between two small beakers. The content of one beaker is used to rinse the mouth thoroughly for one minute, especially interdentally. The oral content is collected in a suitable receptacle and filled up to 7 to 8 c.c. with the subject's saliva. The procedure is repeated with the second part of the glucose solution, the total time necessary being 5 to 10 minutes. Polarimetric analysis showed that on an average 90 per cent of the glucose given was recovered.

The two glucose-saliva mixtures are poured into a thick-walled test tube and carefully homogenized by rolling. From the lower part 6 c.c. is aspirated by pipette and distributed between two plastic tubes (transparent polyvinyl), each containing about 3 c.c. These are stoppered at one end and closed air-free at the other by a clamp. Care must be taken that superfluous saliva is washed away by a jet of distilled water. Another 3 c.c. of the mixture is used at once for titrating initial acidity with 0.01 N NaOH and bromthymol blue, with the usual precautions, to pH 6.6. This must be completed as quickly as possible.

For aerobic fermentation, the remaining 6 c.c. is poured into two open Petri dishes presenting a large surface to air.

The dishes and the plastic tubes are incubated at 37° C. One hour after rinsing they are titrated, after addition of 0.05 c.c. per cubic centimeter of 0.1 per cent alcoholic bromthymol blue.

The change of color may be compared with a set of identical tubes filled with standard buffer mixtures. The turbidity of saliva may be imitated by adding a small amount of starch.

As duplicate tests agreed, only single tests were run.

If paraffin-stimulated saliva is used, the saliva is mixed with an equal amount of the glucose solution immediately after collection and then processed further in the way just described.

RESULTS

After about 200 preliminary tests, systematic examination was set up. First, a series of about 150 measurements was made with saliva from six subjects. As an example of the results, Table I reports twenty-four tests in subject No. 1.

These figures clearly show that the production of acid at low pO_2 always surpassed that at higher pO_2.

The results in the other five subjects were very similar. In Table II we report only the average values, as well as the minimum and maximum values. When the experiments were extended to a greater number of subjects, two kinds of difficulties were met: some subjects were not able to secrete enough saliva, and others produced a mixture acidifying so rapidly that most of the acids had

TABLE I.*

TITRATION ACIDITY OF SALIVA IN SUBJECT NO. 1

TEST NUMBER	ACIDITY AFTER 10 MINUTES (a)	ACIDITY AFTER 30 MINUTES			ACIDITY AFTER 60 MINUTES		
		AEROBIC (b)	ANAEROBIC (c)	DIFFERENCE (c-b)	AEROBIC (d)	ANAEROBIC (e)	DIFFERENCE (e-d)
1	6	7	24	17	10	33	23
2	0	0	14	14	3	19	16
3	− 8	−13	6	19	−13	13	26
4	− 4	− 8	3	11	−10	11	21
5	14	15	24	9	21	30	9
6	4	0	17	17	− 3	23	26
7	−10	−18	4	22	−15	7	22
8	0	2	18	16	4	28	24
9	0	0	13	13	− 6	18	24
10	0	0	5	5	3	20	17
11	0				− 3	16	19
12	0				− 7	13	20
13	10				− 6	16	22
14	8				−14	24	38
15	1				−10	8	18
16	6				3	23	20
17	0				− 5	11	16
18	0				− 9	10	19
19	0				−10	2	12
20	5				− 3	29	32
21	8				7	20	13
22	8				− 3	24	27
23	2				−10	4	14
24	5				− 2	23	25
Average (approximate)	2.5	− 1.5	13	14.5	− 3.5	18	21.5

*In all of the tables in this article the figures of the number of cubic centimeters needed for neutralization of 1 c.c. mixture are multiplied by 100. If the final pH was higher than 6.6, titration was done with 0.01 N H_2SO_4. These figures are marked with a negative sign.

TABLE II

TITRATION ACIDITY OF SALIVA IN SUBJECTS NO. 2 TO NO. 6

SUBJECT NUMBER	RESULTS	ACIDITY AFTER 10 MINUTES (a)	ACIDITY AFTER 30 MINUTES			ACIDITY AFTER 60 MINUTES		
			AEROBIC (b)	AN-AEROBIC (c)	DIFFERENCE (c-b)	AEROBIC (d)	AN-AEROBIC (e)	DIFFERENCE (e-d)
2	average	9	8	16	8	9	23	14
	maximum	20	13	20		25	37	
	minimum	0	− 3	9		− 3	11	
3	average	10	7	17	10	11	26	15
	maximum	24	16	26		34	46	
	minimum	0	0	8		− 5	12	
4	average	9	10	19	9	14	26	12
	maximum	19	12	28		24	43	
	minimum	0	6	13		9	18	
5	average	22	22	27	5	32	41	9
	maximum	41	28	34		52	62	
	minimum	10	13	21		18	32	
6	average	− 1	− 6	7	13	1	23	22
	maximum	10	0	11		42	60	
	minimum	− 6	−22	0		−28	0	
Average (approximate)		10	8	17	9	13.5	28	14.5

TABLE III

TITRATION ACIDITY OF SALIVA IN SUBJECT NO. 7-NO. 61

NUMBER OF SUBJECTS	NUMBER OF TESTS	METHOD	RESULTS	ACIDITY AFTER 0 MINUTES (a)	ACIDITY AFTER 10 MINUTES			ACIDITY AFTER 60 MINUTES		
					AEROBIC (b)	ANAEROBIC (c)	DIFFERENCE (c-b)	AEROBIC (d)	ANAEROBIC (e)	DIFFERENCE (e-d)
15	40	Rinsing with a solution of glucose	average		11			14	29	15
			maximum		36			75	106	
			minimum		–20			–30	1	
40	75	Chewing paraffin	average	– 9	–15*	– 8*	7	– 3	8	11
			maximum	18	20	20		46	55	
			minimum	–43	–29	–22		–43	–31	

*This test was taken on a limited number of subjects only.

already been formed before the test started. Therefore, we were obliged to use paraffin-stimulated saliva. In this way the acidity could be measured immediately after adding the glucose solution.

Experimental results fully agreed with those in Tables I and II: the production of acid from glucose-saliva mixtures was always increased under hypoxic conditions.

Table III reports experiments with subjects from whom saliva was collected in both ways, i.e., by rinsing or by stimulation.

The figures show that the average difference between c.c. 0.01 N NaOH, necessary for neutralization under aerobic and anaerobic circumstances after sixty minutes, was approximately 0.14. By chewing paraffin, anaerobic acid production was three times that under aerobic conditions (compare figures of average results d and e with a).

DISCUSSION

It has been shown in 270 experiments with sixty-one subjects that production of acids in glucose-saliva mixtures, kept at 37° C. for one hour, depends upon the pO_2 of the mixture. At low pO_2 the production is much increased. This may be caused by the difference in lactobacilli growth relative to the pO_2 of the salivary medium,[2, 4] as well as by the preponderance of bacterial glycolysis at low oxygen tensions.

It is possible that acidification under hypoxic circumstances could occur in vivo, too, causing, for instance, a difference in salivary acidity during sleep in subjects breathing with closed or open mouth. In places where carious dentin is covered by a layer of enamel, the pH also might decrease hypoxically. Further research has to take this into account.

SUMMARY

Acidification in glucose-saliva mixtures is, in addition to other factors, dependent upon the oxygen tension of the mixture. In relevant experiments the question as to whether there is more or less contact with air should not be neglected.

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THE ESTIMATION OF SAMPLE SIZE IN EXPERIMENTS
II. USING COMPARISONS OF PROPORTIONS

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IN THE first article of this series methods were described for estimating the size of samples for experiments in which the average readings of two series of measurements were compared. It is the purpose of this article to describe technics for estimating sample sizes in experiments in which proportions are to be compared. As in the first part, before attempting to estimate sample sizes needed for statistically significant differences, the investigator needs to have a certain amount of preliminary data and information as to what contemplated result is considered important.

One of the experimental designs occasionally encountered in dental research involves the comparison of a proportion (p) (usually a percentage) obtained from the study of a sample group, with a generally accepted proportion (universe proportion, p′). For example, the percentage of children caries-free at a certain age, living since birth in an area with 1.0 ppm of fluoride in the drinking water, might be compared with the universe percentage of children of the same age caries-free, from nonfluoride areas.[1] Thus, studies of the dental status of many New Jersey children, age 9 at last birthday, disclosed that 29 per cent were caries-free (p′). Examinations of twenty-five children, age 9, from fluoride areas might show that 10 or 40 per cent (p) were caries-free.

The difference between the percentage of children caries-free from fluoride and nonfluoride areas then would be tested for statistical significance. The following procedure usually is employed. Percentages in repeated samples of twenty-five children from fluoride-free areas are distributed in a normal curve centered at p′ (= 29%), with a standard deviation given by the formula:

$$\sigma_p = \sqrt{\frac{p'q'}{n}}$$

where p′ is the universe percentage of children caries-free, q′ (= 100% − p′) is the universe percentage of children with caries, and n is the size of the sample studied. Thus:

$$\sigma_p = \sqrt{\frac{(29)\ (71)}{25}} = \sqrt{82.4} = 9.1\%$$

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The comparison of the sample from the fluoride area with the universe proportion is made in terms of

$$\text{Dev}/\sigma = \frac{p - p'}{\sigma_p}$$

where the sign of $(p - p')$ is not taken into account here.

$$\text{Dev}/\sigma = \frac{40\% - 29\%}{9.1\%} = 1.2$$

From standard tables of the normal curve,[2] we find that the probability of equaling or exceeding a difference in percentages corresponding to $\text{Dev}/\sigma = 1.2$ is 23 per cent because of chance factors alone. The difference between the percentages of children caries-free in the fluoride and nonfluoride areas is termed "statistically not significant." The null hypothesis being tested, that there is no difference between the percentages of children age 9, caries-free, from these two areas therefore cannot be rejected.

While the difference in the percentage of children caries-free is not significant, nevertheless, the investigator may feel that it is suggestive. He may feel that if he were to examine a larger group of 9-year-olds from fluoride areas, the difference would become statistically significant. The question of prime importance, then, is, How many children should be examined?

As more children are examined, the percentage of caries-free individuals will not necessarily continue to be 40 per cent. The true percentage for fluoride areas is expected to be included within the 95 per cent confidence range,

$$40\% \pm 2\sigma_p = 40\% \pm 2\ (9.1\%)$$

or

21.8% to 58.2%*

The use of this confidence range may serve as a guide to what true percentage of children caries-free in fluoride areas (and thus, what true difference in percentages) it might be important or feasible to expect. Once the true percentage to be focused upon has been chosen, the size of the sample necessary in a larger, more definitive study can be estimated by the following method.

Let us suppose that the true percentage chosen is 50 per cent, so that the true difference between the fluoride and fluoride-free areas is 50% - 29% = 21%. The investigator next must decide what level of significance he should use, e.g., 1 per cent or 5 per cent (maximum probability of error of the first kind), and how large a chance he wants to take of having the difference not be statistically significant when, in fact, the true difference is 21 per cent, an error of the second kind. He may want to take this chance 1 or 5 per cent of the time so that the power of the test would be 99 or 95 per cent, respectively. Let us take the 1 per cent level of significance with 99 per cent power. The question can then be framed as: "If the true difference between the percentages of 9-year-olds, caries-free, from fluoride areas and nonfluoride areas, is really 21 per cent, how many cases should be studied so that the probability is 99 per cent that the obtained difference in the percentages $(p - p')$ will be statistically significant at the 1 per cent level?"

*More precisely, from 21.1% to 61.3%.[3]

In the normal curve of percentages centered at 29 per cent, the upper 1 per cent of the area of the curve is found to the right of $29\% + 2.33\sqrt{\frac{(29\%)\ (71\%)}{n}}$. In the normal curve of percentages centered at 50 per cent, the lower 1 per cent of the area is found to the left of $50\% - 2.33\sqrt{\frac{(50\%)\ (50\%)}{n}}$. The difference between 50 per cent and 29 per cent thus must equal the sum of

$$2.33\sqrt{\frac{(29\%)\ (71\%)}{n}} \text{ and } 2.33\sqrt{\frac{(50\%)\ (50\%)}{n}} \qquad \text{(Fig. 1).}$$

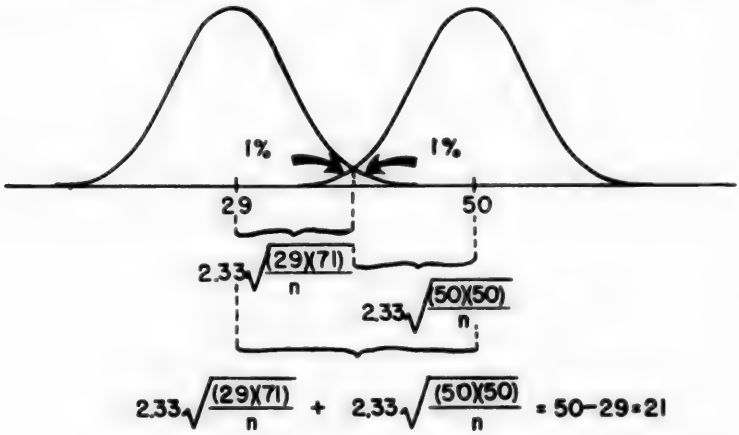

Fig. 1.

Solving for n,

$$2.33\sqrt{\frac{(29\%)\ (71\%)}{n}} + 2.33\sqrt{\frac{(50\%)\ (50\%)}{n}} = 21\%$$

$$\frac{2.33}{\sqrt{n}}\left[\sqrt{(29\%)\ (71\%)} + \sqrt{(50\%)\ (50\%)}\right] = 21\%$$

$$\sqrt{n} = \frac{2.33}{21\%}\left[\sqrt{(29\%)\ (71\%)} + \sqrt{(50\%)\ (50\%)}\right]$$

$$\sqrt{n} = \frac{2.33}{21\%}\ (45.38\% + 50.00\%) = 10.58$$

$n = 112$ children in the sample from fluoride areas.

If the investigator desired to use the 1 per cent level of significance with a power of 95 per cent, the equation to be solved would be:

$$2.33\sqrt{\frac{(29\%)\ (71\%)}{n}} + 1.64\sqrt{\frac{(50\%)\ (50\%)}{n}} = 21\%$$

so that n would be eighty children in the sample.

Although occasionally we may find a situation in which a sample proportion is to be compared to a universe proportion, such universe values are

rather infrequent. The much more common case is that in which proportions obtained from two separate samples are compared. The data resulting from this type of experiment are often summarized in a fourfold table. For example, the proportion of cases in which infected root canals became sterile after medication with a standard root canal antiseptic, such as formocresol, may be compared with the corresponding proportion for penicillin (based upon Ostrander, Crowley and Dowson[4]). Thirty comparable cases are treated, half with one drug and half with the other, and the results tabulated in a fourfold table (Table I).

TABLE I.
PRELIMINARY STUDY OF EFFECTS OF MEDICAMENTS ON STERILITY OF ROOT CANAL CONTENTS

DRUG	STERILE	NOT STERILE	TOTAL	PER CENT STERILE
Formocresol	9	6	$15 = n_1$	$60 = p_1$
Penicillin	5	10	$15 = n_2$	$33.3 = p_2$
Total	14	16	30	$46.7 = p_0$

The difference in the percentages becoming sterile ($p_1 - p_2$) is 26.7 per cent. While this difference appears large, it is statistically not significant, as may be seen by comparison with the standard error of the difference.

$$\sigma_{p_1 - p_2} = \sqrt{\sigma^2_{p_1} + \sigma^2_{p_2}}, \text{ where}$$

$$\sigma^2_{p_1} = \frac{p_0 q_0}{n_1} = \frac{(46.7\%)\ (53.3\%)}{15}, \text{ and}$$

$$\sigma^2_{p_2} = \frac{p_0 q_0}{n_2} = \frac{(46.7\%)\ (53.3\%)}{15}, \text{ so that}$$

$$\sigma_{p_1 - p_2} = \sqrt{165.88 + 165.88} = \sqrt{331.76} = 18.2\%$$

In the normal curve of differences in percentages centered at 0 per cent, we find

$$\text{Dev}/\sigma = \frac{(p_1 - p_2) - 0}{\sigma_{p_1 - p_2}} = \frac{26.7\%}{18.2\%} = 1.47^*$$

While this difference of 26.7 per cent in successful results is statistically not significant, it certainly appears suggestive, so that the investigator might like to know how many cases in each group would be needed for significance to be asserted. The 95 per cent confidence range is approximately from 26.7% + 2(18.2%) and 26.7% − 2(18.2%), or from less than 0 to 63.7 per cent. Suppose that we want to know how many cases are necessary for significance to be asserted at the 5 per cent level with a power of 95%, when the true difference between the percentages of successful cases is, say, 30 per cent in favor of the formocresol medication. Let us take the true value for the formocresol group to be 60 per cent, the value found in the preliminary study. It then follows that the true value for the penicillin medication is assumed to be 30 per cent, and the average for the two medications is 45 per cent. The following procedure will afford an estimate of the number of cases to be studied in each group under the above conditions.

*For small samples, it is often advisable to make a "correction for continuity."[5]

In the normal curve of differences in percentages centered at 0 per cent, the upper 5 per cent of the curve is found to the right of

$$0\% + 1.64\sqrt{\frac{(45\%)\,(100\% - 45\%)}{n_1} + \frac{(45\%)\,(100\% - 45\%)}{n_2}}.$$

In the normal curve of differences in proportions centered at the assumed true difference of 30 per cent, the lower 5 per cent of the area is found to the left of

$$30\% - 1.64\sqrt{\frac{(60\%)\,(100\% - 60\%)}{n_1} + \frac{(30\%)\,(100\% - 30\%)}{n_2}}.$$

The total distance between 30 per cent and 0 per cent must equal the sum of

$$1.64\sqrt{\frac{(45\%)\,(55\%)}{n_1} + \frac{(45\%)\,(55\%)}{n_2}} \text{ and } 1.64\sqrt{\frac{(60\%)\,(40\%)}{n_1} + \frac{(30\%)\,(70\%)}{n_2}}$$

(Fig. 2).

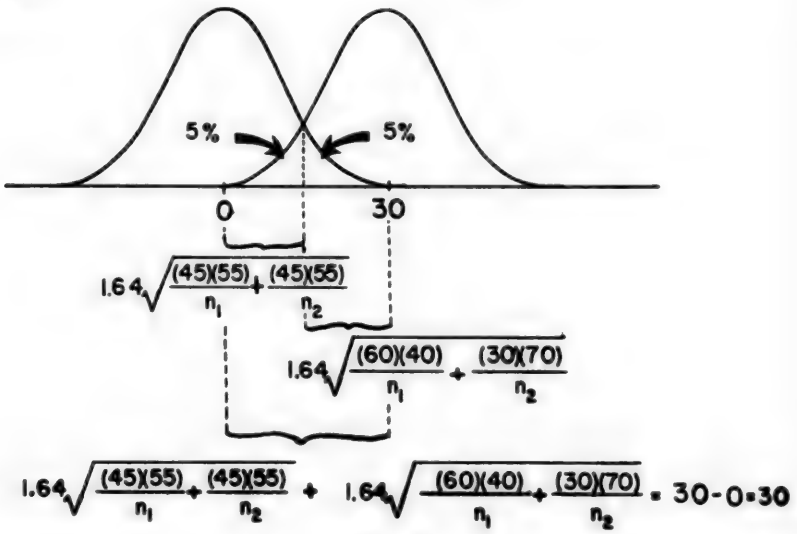

Fig. 2.

Since we are taking the sample size of each group to be equal ($n_1 = n_2 = n$),

$$30\% = 1.64\sqrt{\frac{(2)\,(45\%)\,(55\%)}{n}} + 1.64\sqrt{\frac{(60\%)\,(40\%)}{n} + \frac{(30\%)\,(70\%)}{n}}.$$

Simplifying, and solving for n,

$$\sqrt{n} = \frac{1.64}{30\%}\left[\sqrt{(2)\,(45\%)\,(55\%)} + \sqrt{(60\%)\,(40\%) + (30\%)\,(70\%)}\right]$$

$$\sqrt{n} = \frac{1.64}{30\%}\,(70.36\% + 67.08\%) = \frac{(1.64)\,(137.44)}{30}$$

$\sqrt{n} = 7.48$, so that n is about fifty-six cases in each group.*

If, instead of the 5 per cent level of significance with a power of 95 per cent, the investigator had chosen the 1 per cent level of significance with 99 per cent power of the test, the equation to be solved would be,

*If the original formocresol percentage were based upon a large enough series of observations so as to be considered the true or universe percentage (p'), the same significance and power would be obtained with only about one-half of the indicated number of cases in the penicillin group (in this instance 28 cases).

$$\sqrt{n} = \frac{2.33}{30\%}\left[\sqrt{(2)\,(45\%)\,(55\%)} + \sqrt{(60\%)\,(40\%) + (30\%)\,(70\%)}\right]$$

so that n is about 114 cases in each group. It will be noted that the size of the sample in each group necessary for significance to be asserted at the 1 per cent level, 99 per cent power, is just about twice the number of cases necessary at the 5 per cent level and 95 per cent power. This follows from the fact that the ratio of $\frac{(2.33)^2}{(1.64)^2}$ is almost exactly 2.

TABLE II*

ESTIMATED SAMPLE SIZES NECESSARY IN EACH OF TWO GROUPS CORRESPONDING TO VARIOUS ASSUMED TRUE PERCENTAGES IN ORDER TO INSURE SIGNIFICANCE AT THE 1% LEVEL WITH A 99% POWER OF THE TEST

SMALLER TRUE PERCENTAGE	ANTICIPATED TRUE INCREASE IN PERCENTAGE											
	5%	10%	15%	20%	25%	30%	35%	40%	45%	50%	55%	60%
5%	1,116	365	191	122	86	65	55	42	35	29	25	21
10%	1,878	539	266	163	112	82	63	50	41	34	29	24
15%	2,500	683	327	196	131	95	72	56	45	37	31	26
20%	3,002	803	377	221	147	104	78	61	48	39	32	26
25%	3,448	904	417	241	158	111	82	63	50	39	32	26
30%	3,795	980	447	255	165	115	84	64	50	39	31	24
35%	4,055	1,035	466	264	169	117	84	63	48	37	29	21
40%	4,225	1,067	476	267	169	115	82	61	45	34	25	
45%	4,314	1,078	476	264	165	111	78	56	41	29		
50%	4,314	1,067	466	255	158	104	72	50	35			
55%	4,225	1,035	447	241	147	95	63	42				
60%	4,055	980	417	221	131	82	55					
65%	3,795	904	377	196	112	65						
70%	3,448	803	327	163	86							
75%	3,002	683	266	122								
80%	2,500	539	191									
85%	1,878	365										
90%	1,116											

*For computational convenience, the values in this table were calculated by the arc sine transformation of percentages.[7]

The methods just described for estimating sample sizes, given the anticipated true difference, the respective true percentages upon which the true difference is based, and the desired significance level and power of the test, have been utilized in the construction of Table II. This table shows immediately, for any given choice of the true percentage in one group and the anticipated true difference between percentages, the sample size necessary in each group corresponding to the 1 per cent significance level and 99 per cent power of the test. Thus, if the true percentage of infected root canals becoming sterile after routine medication is assumed to be 45 per cent, and a new drug is assumed to increase the per cent becoming sterile by 30 per cent, the table shows us in the cell corresponding to 45 per cent (vertical scale) and 30 per cent (horizontal scale), the number of cases for each group, namely 111.

If the 5 per cent level of significance and 95 per cent power of the test should be desired, it is merely necessary to divide the corresponding number in the table by 2. If different significance levels and/or powers are desired, it is necessary to resort to appropriate substitutions for the critical values

1.64 and 2.33 in the equations developed in the body of this article. A nomogram giving sample sizes for various significance levels and powers has been constructed and can also be utilized for this purpose.[6] In the event that the percentage for one universe is actually known (p′) so that a sample is necessary only in the other group, the necessary size in the latter group is approximately one-half the number indicated in the table.

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THE CLASSIFICATION OF NONHEMOLYTIC STREPTOCOCCI RECOVERED FROM BACTEREMIA OF DENTAL ORIGIN

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EXTRACTIONS of teeth occasionally give rise to subacute bacterial endocarditis, and we may postulate that the causative organisms originate from regions adjacent to the teeth and that they invade the blood stream in the course of the removal of teeth. Accordingly, we have attempted to compare streptococci isolated from bacteremia after dental extractions with those reported in subacute bacterial endocarditis and in bacteriologic studies of the oral cavity.

The organisms recovered most frequently from the blood following dental procedures and during subacute bacterial endocarditis are nonhemolytic streptococci of the alpha and gamma types.[7, 22, 23] The normal habitat of most members of this heterogeneous group is the oral cavity and the throat.

Some nonhemolytic streptococci possess a group-specific C substance and can be classified, together with homologous hemolytic streptococci, under Lancefield Groups A to O. The majority of nonhemolytic streptococci are designated as "viridans."[5] Most of these have not yet been adequately defined for identification and are tentatively identified on the basis of physiologic characteristics. Recently three species have been more fully described, and their association with disease entities have been elucidated. They are characterized serologically by their reactions with type-specific antigens.

Streptococcus MG, an inhabitant of the normal mouth repeatedly associated with primary atypical pneumonia, has been shown to elaborate a type-specific capsular polysaccharide.[16] It can be identified by a quellung reaction with specific MG antiserum and by a unique pattern in biochemical tests.

Streptococcus sanguis, reported by Loewe, Candel, and Eiber,[14] as the nonhemolytic streptococcus most commonly recovered from 145 cases of subacute bacterial endocarditis, has been shown to be relatively homogeneous in physiologic tests and antigenic structure.[13, 34] One of the distinguishing characteristics of *Str. sanguis* is its ability to synthesize large amounts of dextran from sucrose. Loewe, Plummer, Niven, and Sherman,[13] working with 680 isolates from 20 healthy individuals, found no *Str. sanguis* in the throats of the subjects. In contrast to these results, Hehre[10] studied 50 to 70 isolates from each of 18 healthy adults for dextran synthesis. Twenty-eight strains from 9 of the subjects produced abundant dextran and showed biochemical reactions similar to those of *Str. sanguis.* Hehre and Neill[9] found 9 strains of Group H streptococci which were strong dextran producers. *Str. sanguis* strains studied

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by Washburn, White, and Niven[34] were checked only against streptococcus antisera of Lancefield Groups A to G, so that it is open to conjecture whether any belonged to Group H.

Streptococcus salivarius, another well-defined viridans streptococcus, is a normal inhabitant of the oral cavity. Since first described by Andrewes and Horder in 1906,[1] it has been reported as a frequent causative organism in subacute bacterial endocarditis. Since Sherman, Niven, and Smiley[29] limited its definition to those viridans streptococci which produce large mucoid colonies on 5 per cent sucrose agar, it has rarely been reported as a pathogen.[14, 24] With serologic tests, a Type I, a Type II, and probably other unknown type-specific antigens are found among *Str. salivarius.*

In order to compare our microorganisms with those described in the oral cavity and with those mentioned in reports on subacute bacterial endocarditis, we used all methods available for taxonomic identification. Selbie, Simon, and Robinson[25] and Solowey[31] approached the comparison of oral and cardiac streptococci without actually identifying the various species. They based their serologic studies on the type-specific substances contained in nonhemolytic streptococci.

Selbie, Simon, and Robinson[25] completed a serologic classification of 96 strains from endocarditis cases, 103 strains from the throat and teeth, and 10 from other sources. Antisera were prepared against 8 strains of streptococci from endocarditis cases, including one *Str. sanguis* strain, and these were tested by the precipitin technic against extracts from the 209 strains. These workers concluded that nonhemolytic streptococci isolated from cases of subacute bacterial endocarditis produced a serologic pattern similar to those isolated from the teeth and throats. Their findings were in agreement with those of Solowey[31] who charted the cross reactions between antisera and extracts from 107 endocarditis strains and 98 strains from the human throat and extracted teeth. These studies suggest a similarity between the nonhemolytic streptococci in the mouth and those isolated from the blood of endocarditis patients. However, Selbie, Simon, and Robinson[25] stated that "the largest group in streptococci from teeth and throats have less tendency to produce s.b.e. than the streptococci of the other four groups."

METHODS

At the beginning of this study serologic groupings were done in our own laboratory following Lancefield's precipitin technic.[32] Since the majority of antisera became difficult to obtain, a large number of the strains were grouped at the Streptococcus Laboratory, Communicable Disease Center, Chamblee, Ga. We used streptococcus MG antiserum for the quellung reaction with those streptococci which exhibited the biochemical characteristics of streptococcus MG. Specific antisera for *Str. salivarius* and *Str. sanguis* could not be obtained.

Among the various physiologic tests reported for the differentiation of aerobic or facultatively anaerobic streptococci, we selected those that gave consistent results when tested against known stock cultures. Hemolysis was

determined from poured horse blood agar plates. All tests were matched with appropriate controls. The following is a partial list of references for the procedure employed in this study: Resistance to 60° C. for 30 minutes (Shattock and Mattick[26]), hydrolysis of sodium hippurate (Hare and Colebrook,[8] Coffey and Foley[4]), hydrolysis of arginine (Niven, Smiley, and Sherman[19]), starch hydrolysis (Hodgson, Heggie, and Sutherland[11]), liquefaction of gelatin (Mirick, Thomas, Curnen, and Horsfall[16]), splitting of esculin (Mirick, Thomas, Curnen, and Horsfall[16]), slime formation in 5 per cent sucrose broth (Niven, Kiziuta, and White[20]), mucoid colonies on Niven's agar (Niven, Smiley, and Sherman[18]), and growth on 10 per cent and 40 per cent bile blood agar (Belenky and Popowa[2]).

RESULTS

In our studies of blood cultures taken immediately after extractions from ambulatory patients of Tufts College Dental School, a high number of cases were found positive for nonhemolytic streptococci. One hundred sixty strains have been classified following Swift's chart.[5] Based on Sherman's work, the entire genus *Streptococcus* is divided into four divisions: (1) Hemolytic—Lancefield Groups A, B, C, E, F, G, H, K, L, M, and O. (2) Viridans. (3) Enterococci—Group D. (4) Lactic—Group N.

Of the total of 160 strains* of nonhemolytic streptococci classified, 47 (29.4 per cent) were found to belong to Lancefield Groups A to O. Thirty-one of these were of Group K. Eight belonged to Group F, 4 to Group H, and only 1 or 2 fell into each of Groups A, C, and L. Three strains reacted with both F and K antisera and were tabulated in the group which better fitted their biochemical reactions. One strain grouping as L in our laboratory was reported by the Streptococcus Laboratory as reacting with F, K, and L antisera. No explanation for these multiple reactions was offered, but some discrepancy might be expected in testing alpha-hemolytic strains against antisera prepared with beta-hemolytic strains.

In the viridans group the most common species found was *Streptococcus mitis* which made up 47.5 per cent of the 160 classified strains. Difficulty was encountered in fitting the other streptococci into the better-defined species. One strain was classified as *Streptococcus thermophilus* because it agreed with the definition of this species in all but the fermentation of maltose. Two strains were classified as *Streptococcus equinus*, even though one would not grow at 45° C. and one hydrolyzed sodium hippurate. Neither fermented lactose, both grew on 40 per cent bile blood agar and split esculin, and neither produced any reaction in litmus milk with 2 per cent dextrose added. Two strains were classified as streptococcus MG on the basis of biochemical tests and a positive quellung reaction with specific antiserum.

Five strains were similar in many properties to *Str. sanguis*. They can be classified only tentatively, since no specific antisera were available for verification through serologic tests. Three additional strains were similar to *Str. sanguis* in hydrolyzing arginine and gelling 5 per cent sucrose broth, but

*Representative strains have been preserved by freeze-drying.

TABLE I

CLASSIFICATION OF 160 STRAINS OF NONHEMOLYTIC STREPTOCOCCI RECOVERED FROM BACTEREMIA OF DENTAL ORIGIN

SPECIES	NUMBER OF STRAINS	HEMOLYSIS	RESISTS 60° C. FOR 30 MINUTES	GROWTH 10° C.	GROWTH 45° C.	GROWTH 6.5% NaCl BROTH	HYDROLYSIS OF HIPPURATE	HYDROLYSIS OF ARGININE	HYDROLYSIS OF STARCH	FERMENTATION OF LACTOSE	FERMENTATION OF MALTOSE	FERMENTATION OF TREHALOSE	FERMENTATION OF RAFFINOSE	FERMENTATION OF INULIN	FERMENTATION OF GLYCEROL	FERMENTATION OF SORBITOL	FERMENTATION OF ESCULIN	SLIME IN 5% SUCROSE BROTH	MUCOID COLONIES ON NIVEN'S AGAR	GROWTH ON BILE BLOOD AGAR BILE PER CENT 10	GROWTH ON BILE BLOOD AGAR BILE PER CENT 40	REMARKS
Group A	72	G	–	–	–	–	–	+	–	+	+	±	–	–	–	–	+	–	–	±	–	
C	1	A	–	–	+	–	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–	
F	8	A*	–	–	±	–	–	±	–	+	+	±	–*	–	–	–	±	–	–	±	±	1 gamma and 1 beta hemolysis 2 strains react with K
H	4	A	±	–	±	–	–	±	–	±	+	±	±	±	–	–	±	–	–	–	–	
K	31	A*	–	–	±	–	–	±	–	±	+	±	±	–■		–	±	–	–*	±	–	1 gamma hemolysis 1 strain reacts with F
L	1	A	–	–	–	–	–	+	+	+	+	–	+	–	–	–	+	sl	–	–	–	L in our lab. Chamblee—K, F, L.
Equinus	2	A	±	–	±	–	±	–	–	–	+	+		–	–	–	±	–	–	+	+	no reaction in litmus milk + 2 per cent dextrose
MG	2	G	–	–	–	–	–	+	–	+	+	+	–	–	–	–	+	–	–	+	+	positive quellung with specific MG antiserum
Mitis	76	A G	–	–	±	–	–	±	–	±	+	±	±	–	–	–	±	–	–	±	–	
Salivarius	5	A G	–	–	±	–	–	–	–	+	+	±	±	±	–	±	±	–	halo sl mucoid	–	–	pH 4.2-4.4. Reduced litmus after clotting
Sanguis	5	A	–	–	±	–	–	+	–	+	+	+	–	+■	–	–	+	sl to heavy	–	±	–	
Sanguis?	3	A	–	–	±	–	–	+	–	+	+	+	+	–	–	–	+	+	–	±	–	
Thermophilus	1	A	+	–	+	–	–	–	–	+	+	–	+	–	–	–	–	–	–	–	–	no growth in 2% NaCl broth
Unid. #1	10	A*	+	–	+	–	–	–	–	+*	+	±	±	–*	–	–	–■	–	–	–	–	
Unid. #2	2	A	+	–	+	–	–	+	–	+	+	±	–	–	–	–	+	–	–	+	+	reduced litmus after clotting, not liquefying gelatin
Unid. #3	3	A G	–	–	–	–	–	+	–	+	+	+	–	–	–	–	+	–	–	+	+	no quellung reaction with MG antiserum
Unid. #4	1	A	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	
Unid. #5	3	A	–	–	±	–	–	–	–	+	+	–	+	+	–	–	–	–	–	–	–	

*Minor exceptions.
A—Alpha hemolysis.
G—Gamma hemolysis.
Unid.—Unidentified strains.
Sl—Slight.

differed in that they fermented raffinose and did not ferment inulin. In the absence of *Str. sanguis* antiserum these 3 might be classified either as *Str. mitis* or *Str. sanguis*, since some *Str. mitis* strains also synthesize dextran from sucrose.[35]

Five strains were similar to *Str. salivarius*. Although all colonies on 5 per cent sucrose agar were surrounded by halos, they were small and only slightly mucoid. The one strain in our collection which most resembled a typical *Str. salivarius* was not included in this group because it reacted strongly with Group K antisera. The reaction was verified at the Streptococcus Laboratory. This strain produced large mucoid colonies on 5 per cent sucrose agar, produced gamma hemolysis in blood agar, fermented inulin, raffinose, and esculin, did not ferment lactose, and grew at 45° C. and on 10 per cent bile blood agar.

Among the 19 strains listed as unidentified, 10 strains were fairly homogeneous in their ability to resist 60° C. for 30 minutes, and grow at 45° C. and in 2 per cent NaCl broth, and in their lack of growth on 10 per cent bile blood agar. Of the remaining 9, 2 strains were similar to the enterococci in that they resisted 60° C. for 30 minutes, grew at 45° C., hydrolyzed arginine, and grew on 40 per cent bile blood agar, but differed in that they reduced litmus only after clotting and were unable to grow at 10° C. or in 6.5 per cent NaCl broth. Three strains hydrolyzed arginine and grew on 40 per cent bile blood agar, but did not grow at 45° C. and produced no quellung reaction with streptococcus MG antisera. One strain consistently withstood 60° C. for 30 minutes, but did not grow at 45° C. or on 10 per cent bile blood agar. The remaining 3 strains were similar to *Str. mitis* except for the fermentation of inulin.

DISCUSSION

Since Burket and Burn[3] have shown that bacteria from the oral cavity may enter the blood stream during dental extractions, we might expect that at least part of the streptococci classified in our study had their origin in the oral cavity.

Among the streptococci in the mouth and throat, *Str. salivarius* and *Str. mitis* are the most commonly reported. Sherman, Niven, and Smiley[29] studied 331 cultures of nonhemolytic streptococci isolated from the throats of normal persons and identified 184 strains as *Str. salivarius* and 147 strains as *Str. mitis*. Hehre[10] in studying 1,096 isolates from cultures of normal throats found 296 levan producers of which 20 representative strains were typical of *Str. salivarius*. It would appear from these two papers that *Str. salivarius*, even under its stricter definition, is still one of the more common nonhemolytic streptococci. It is surprising, then, that we found such a small number of *Str. salivarius* in contrast to the large number of *Str. mitis*. Most of the remaining species that we have identified have been reported to occur in the mouth, but their average distribution among the oral flora is not known.

The major studies on the species of streptococci recovered from the blood of patients with subacute bacterial endocarditis are listed in Table II. *Str.*

mitis has been isolated in about 23 per cent of the cases. The organism has been found twice as often in our bacteremia study, but it constitutes a group which is too heterogeneous to be used as a basis for comparison. The most homogeneous group of viridans streptococci, *Str. salivarius,* has been mentioned as causative agent in 9 per cent of the cases, but not all investigators followed Sherman's definition of the species and, furthermore, not all of the strains were isolated from the blood of the patients.[30] The only well-defined organism for which comparative data are available is *Str. sanguis.* It has been identified in the blood of 44 per cent of 326 patients with subacute bacterial endocarditis, while we could identify only a small number of strains in our bacteremia studies, and it probably occurs infrequently in the oral cavity. These facts may help to explain why tooth extraction, the most universal surgical intervention, is not followed by infective heart disease more often (Robinson, Kraus, Lazansky, Wheeler, Gordon, and Johnson[23]).

TABLE II

NONHEMOLYTIC STREPTOCOCCI ISOLATED FROM SUBACUTE BACTERIAL ENDOCARDITIS AS REPORTED BY VARIOUS INVESTIGATORS

INVESTIGATOR	TOTAL STRAINS	LANCEFIELD GROUPS		VIRIDANS					
		D	OTHER	MITIS	SALIVARIUS	SANGUIS	BOVIS	EQUINUS	UNCLASSIFIED
Foley[6] Various sources	34	19	K-1	3		6	5		
Loewe, Candel, and Eiber[14] Jewish Hospital, Brooklyn	145	6	*	23	1	85	13		17
MacNeal and Blevins[15]	31	6	*		13		3	7	2
Moran[17] St. Louis and other cities	20	5	*		12				3
Niven and White[21] Cornell University	113	5	B-4 G-1	45		42	12		4
Schneierson[24] Mt. Sinai Hospital, N.Y.C.	34	2	*		2	9			21
Tumulty and McGehee[33] Johns Hopkins	31	2	*	7	9			2	11

*No data other than Group D.

Our classification results were compared with the clinical data in an attempt to answer three questions: (1) Is streptococcemia caused more often by single strains or by several different strains? (2) Is the blood of patients more likely to harbor repeatedly the same strains of streptococci or different strains? (3) Does the species recovered bear any likely relationship to the medical history of the patient?

The 160 classified strains were cultured from the blood of 78 patients following 94 operations. The majority of bacteremia (Table III) seemed to be caused by single strains of streptococci. However, this majority becomes insignificant if we consider how easy it is to overlook different strains whose colony forms are indistinguishable. Therefore, we may assume that there is no predominance of single-strain bacteremia.

Sixty-five patients were operated on once, while 13 patients underwent 2 to 4 operations accompanied by bacteremia. From 10 of these latter patients, 32 strains were recovered following 21 operations. Only from the re-

maining 3 patients were identical strains obtained more than once. Even among these patients the occurrence of identical strains followed a chance pattern. For example, one patient showed bacteremia after 4 different operations. If we assign a letter of the alphabet to each strain identified, the 4 bacteremia contained ABC, AD, B, and E strains respectively. From this it seems safe to infer that specific streptococcal strains in bacteremia of dental origin are not characteristic for the individual patient.

TABLE III

THE FREQUENCY WITH WHICH ONE OR MORE STRAINS OF STREPTOCOCCI WERE DETECTED IN THE BLOOD OF SEVENTY-EIGHT PATIENTS FOLLOWING EXTRACTION OF TEETH

NUMBER OF STRAINS	NUMBER OF OPERATIONS	TOTAL STRAINS
1	53	53
2	23	46
3	12	36
4	5	20
5	1	5
Total	94	160

Medical histories were taken with special emphasis on joint and heart disease and on recent bacterial infection. In addition to this, a record was kept of antibiotic medication preceding the operations. There seemed to be a random distribution of species of streptococci with any of the categories investigated.

SUMMARY

One hundred sixty strains of aerobic nonhemolytic streptococci, isolated from the blood of patients after dental extractions, were classified by serologic and physiologic tests. Forty-seven strains were members of Lancefield Groups A, C, F, K, and L. Of the remaining strains, 94 were identified as viridans and 19 could not be classified.

There is little doubt that all of the species recovered occur in the oral cavity. The present knowledge of their proportional distribution in the mouth is too scanty to attempt a comparison with their representation in blood cultures. Although *Streptococcus salivarius* is one of the more common members of the oral flora, we found only a small number in our collection. *Streptococcus sanguis* has been isolated from a high percentage of cases of subacute bacterial endocarditis, yet we have identified few in our bacteremia studies. On the strength of the data available we may conclude that a few of the streptococci recovered from bacteremia of dental origin are potential producers of subacute bacterial endocarditis.

Streptococcemia was frequently caused by 2 or more different strains of organisms. When streptococci were recovered repeatedly from blood samples of the same patient, the strains were rarely identical. The species of streptococci recovered did not show any relationship to the medical history of the patient.

We wish to thank Dr. F. L. Horsfall, Jr., for a strain of streptococcus MG and the specific antiserum, Dr. H. W. Lyall of the New York Department of Health for part of

the grouping antisera, and Dr. E. L. Updyke of the Communicable Disease Center for the grouping of many strains. We wish to express our appreciation to Drs. R. C. Lancefield, H. W. Seeley, and J. M. Sherman for providing us with a complete series of known strains and for giving freely of their advice.

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THE TEMPOROMANDIBULAR JOINT IN VITAMIN C DEFICIENCY

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THE effects of vitamin C deficiency on the teeth, the long bones, and the costochondral junction are well known. There are no published reports, however, describing the changes in the mandibular condyle of animals deficient in vitamin C. The purpose of this article is to describe the histologic changes in the mandibular condyle of scorbutic guinea pigs.

METHOD

Twelve young guinea pigs, each weighing 200 to 300 grams, were placed on a vitamin C-free diet. They were killed after two, three, and four weeks on the deficient diet. A few received injections of ascorbic acid after three weeks on the diet, and were killed three days later. The heads were removed, fixed in formalin, and decalcified by the formic acid-sodium citrate method. Block sections of the mandibular joint were removed, and serial sections for histologic study were prepared.

NORMAL HISTOLOGY OF THE MANDIBULAR JOINT OF THE GUINEA PIG

The condylar head is separated from the fossa by a fibrocartilage disc, which is covered on both sides by a single layer of synovial cells. The condyle is similarly covered by a synovial epithelium, under which is a thin fibrous layer. Beneath this layer is a zone of undifferentiated cells, which look like condensed mesenchyme and which we have called precartilage. This is demarcated from the zone of more differentiated cartilage cells which lies directly beneath it. A zone of hypertrophic cartilage cells extends evenly into the marrow cavity. There are many spicules of bone containing unresorbed calcified cartilage matrix material covered by plump osteoblasts in the marrow cavity. Between the bony spicules a well-vascularized cellular marrow extends to the zone of erosion. Osteoclasts are present at the lateral portions of the head of the condyle, at the junction of the cartilage with the bone, and occasionally at the zone of erosion (Fig. 1).

HISTOLOGY OF THE MANDIBULAR JOINT OF THE SCORBUTIC GUINEA PIG

After two weeks on the vitamin C-deficient diet there is an increase in the number of osteoclasts in the zone of erosion. In the subchondral area there are numerous fibroblast-like cells replacing the hematopoietic cells. Minimal hemorrhage into the marrow cavity is seen (Fig. 2).

After three weeks on the deficient diet there is complete cessation of bone formation. At the zone of erosion, the cartilaginous matrix is calcified. This

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cartilage is separated from the marrow by an acidophilic structureless material. The cartilage is irregular, and there are numerous fractures of the calcified matrix material lying in disarray in the immediate subchondral area. The marrow in this area is replaced by connective tissue cells. There is a reduction in the amount of hematopoietic marrow throughout the ramus, with large numbers of fibroblast-like cells replacing it. In the marrow spaces are numerous fractured spicules of bone surrounded by focal accumulations of multinucleated

Fig. 1.

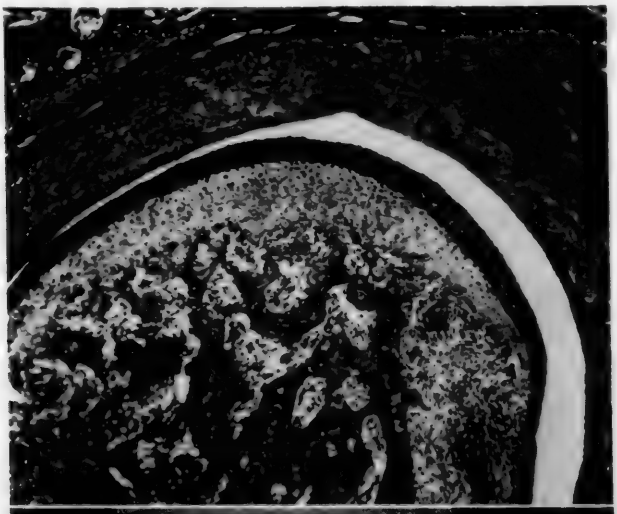

Fig. 2.

Fig. 1.—Photomicrograph of condyle of normal control guinea pig.
Fig. 2.—Photomicrograph of condyle of guinea pig maintained on ascorbic acid-deficient diet for two weeks.

Fig. 3.

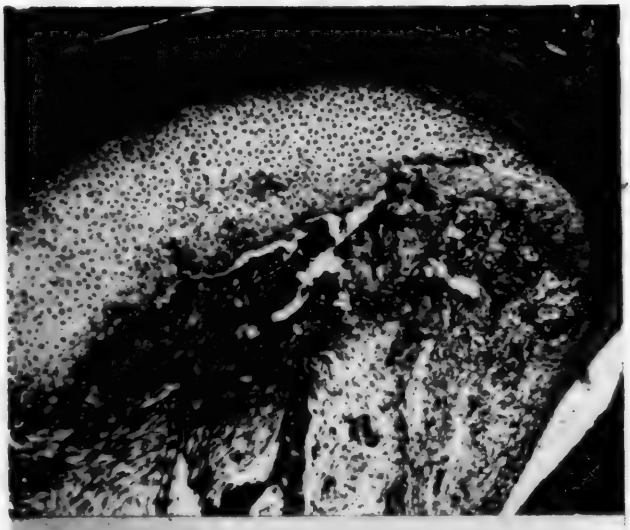

Fig. 4.

Fig. 3.—Photomicrograph of condyle of guinea pig maintained on ascorbic acid-deficient diet for three weeks.

Fig. 4.—Photomicrograph of ramus of mandible of deficient guinea pig after three days of refeeding normal diet.

osteoclasts. The lateral width of the ramus is reduced (Fig. 3). On both sides of the meniscus one finds an acidophilic amorphous material, as well as an occasional red blood cell or lymphocyte.

Another conspicuous change is the edema of the muscle and periosteum. The muscle bundles are loosely grouped, and the individual fibers of the connective tissue are separated from each other. There are small hemorrhages between the muscle bundles, onto and beneath the periosteum, and into the marrow cavity. There is an occasional macrophage containing blood pigment in the edematous periosteum.

After four weeks on the vitamin C-free diet, all of the previously mentioned changes are accentuated further. The edema of the periosteal connective tissue is so marked that it resembles mesenchyme. The subperiosteal hemorrhage is undergoing further resolution, as shown by the laking of red blood cells and the abundance of macrophages. There is now frank hemorrhage into the temporomandibular joint.

Three days following ascorbic acid therapy there is reduction of the edema fluid. Tremendous regrowth of osteophytic bone subperiosteally has increased the lateral ramus width at least threefold. Many of the osteophytes resemble cartilage, especially at the periosteal margin (Fig. 4). There is gradual resolution of the pink-staining structureless material in the subchondral area. The fractures that occurred at the cartilage-shaft junction are being repaired by a large callus formation.

SUMMARY

The histologic changes in the mandibular condyle of animals maintained on vitamin C-deficient diets are described. They are consistent with those described for other bones of scorbutic animals.

A TEST FOR THE PURITY OF POWDERED ENAMEL

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IN A study of the purity of samples of powdered enamel, it was observed that it is difficult to distinguish small fragments of dentin from enamel. In collateral studies it was observed that, while mature human enamel is scarcely affected by the Hotchkiss-MacManus periodic acid-leucofuchsin method,[1] dentin takes on an intense red as a result of this stain. This procedure, suitably modified, should be useful in detecting contaminating dentin in the powdered enamel, and an account of such a method is presented in this paper.

METHOD

Samples of powdered enamel were immersed in 0.5 per cent $HIO_4 \cdot H_2O$ for three minutes and were centrifuged for one minute at 3,000 r.p.m. in a Servall type XL angle centrifuge. The supernatant fluid was decanted and the enamel was washed once with distilled water followed by centrifugation and decantation. The samples then were exposed for thirteen minutes to leucofuchsin prepared by the method described by Lillie.[2] The enamel then was spun down again, and three washings in 0.5 per cent sodium meta bisulfite in 0.1N HCl followed with intervening centrifugations. As a final step, the sulfurous acid was removed with a distilled water rinse followed again by centrifugation. The material remaining could be observed under a dissecting microscope.

Since some regions of the enamel were stained, if somewhat faintly, by the reagents, only those fragments which are clearly dentin should be counted as contaminating material when particle counts are made. Control tests, in which pure dentin is used, are informative.

RESULTS

Dentin fragments are stained a deep red, and the characteristic microscopic appearance of these fragments is emphasized by the red color. As a result of the strongly acid bisulfite rinses, as much as three-fourths of the enamel is dissolved. This amounts to a rather great concentration of the dentin and considerably increases the likelihood of the detection of contaminating material. The rate at which particle counts may be carried out can be increased materially. It is conceivable that this method may have utility in connection with the Manly-Hodge[3] flotation method of preparing enamel, especially where sample purity is a critical issue.

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SUMMARY

Application of the Hotchkiss-MacManus periodic acid-leucofuchsin stain to powdered enamel samples permits ready detection of contaminating dentin fragments. The dentin is stained a deep red by this method, and much of the enamel is dissolved in acid. This results not only in coloration, but also in considerable concentration of the dentin.

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Erratum

In the article by Harold S. Fleming entitled "Effect of Certain Concentrations of Fluoride on Enamel and Dentin as Formed in Transplants of Tooth Germs and Related Studies" which appeared in the August issue of the JOURNAL, the first line of the last paragraph on page 470 should begin, "Ten dba mice used 16 ounces or approximately 500 c.c. of water . . ." instead of 1,250 c.c. On page 482 the ninth line should end, "fluoride ion when" instead of "fluoride on ion when." The thirteenth and fourteenth lines of paragraph 4 on the same page should read, ". . . and many of the step-wise actions . . ." instead of "step-wise of the many actions."

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Proceedings of the Thirty-First General Meeting
Philadelphia, Pa.

March 20, 21, 22, 1953

Compiled by Hamilton B. G. Robinson,* Editor, College of Dentistry,
Ohio State University, Columbus, Ohio

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I. General Information

The Thirty-First General Meeting was held in eight general sessions, four of which were presented in two sections each. At these general meetings a total of 145 papers were presented, and 41 were read by title. The Materials Group coordinated its meeting with the General Meeting and heard 25 papers at four sessions.† At the closing executive meeting Dr. John J. Salley of the University of Rochester received the first Novice Award and Dr. Francis A. Arnold, Jr., delivered his presidential address. Council meetings and the Board of Editors meeting were held during the General Meeting. The meetings of the International Association for Dental Research were attended by 229 members and 352 registered guests.

II. President's Address: Trends in Research

Francis A. Arnold, Jr.

National Institute of Dental Research, Bethesda, Md.

Since the beginning of this Association, some three decades ago, the incoming president has been privileged to address the membership at his inauguration. Some of those speeches will endure through the years as models of erudition and as statements of dental research accomplishments. In the last two years, however, your program committee has wisely planned to bestow the additional virtue of brevity on the inaugural address, so that as much time as possible can be allotted to the more important scientific sessions of our annual meeting. It is this fact—namely, the realization that the philosophical has its place, but that it must follow and derive from the experimental—that furnishes me with the text of this address on "Trends in Dental Research."

*The editor was generously assisted in the preparation of these proceedings by the secretary, Dr. Edward H. Hatton, and by the assistant secretary, Dr. Dan Y. Burrill.

†A new innovation was an early Sunday morning session at which Dr. Joseph Volker spoke on "Science and Religion."

I wish first to express my gratitude for the honor you have bestowed upon me. I can readily remember the occasion, almost twenty years ago, when I attended my first meeting of this Association. I deemed it an honor then to be able to participate in the proceedings of that meeting, and each succeeding meeting has rekindled that feeling. So, today, I can assure you that I appreciate the opportunity and the responsibility of assisting in guiding during the coming year the destinies of the International Association for Dental Research.

Coincidentally with assuming new responsibilities as president of this Association, I have had the good fortune to assume the responsibilities of another office whose objectives are closely allied with those of this organization. I have just this month become director of the Public Health Service's National Institute of Dental Research. The assumption of these two positions of trust has caused me to take stock of myself and to reassess the activities in the field of dental research. I do not have the temerity to predict the future course of dental research, but a review of the past history of this Association and the activities of its members presents certain clear guidelines which have marked the gradual evolvement of our present-day research patterns and may be useful indications of what can be expected in the future.

Anyone attempting even a cursory review of the dental research literature of the past thirty years (as reflected largely in the pages of our own *Journal*) must surely be impressed with the changes that have occurred in this field. While many of the problems are unchanged, to me it is most significant that our approaches to these problems have undergone major reorientation as have, indeed, our attitudes toward research in general.

In the early issues of the *Journal,* considerable space was allocated to philosophical discussions of the purpose and aims of the *Journal* and later of the Association. The scientific reports, which comprised only a portion of each publication, were usually scholarly dissertations on a broad subject of fairly general interest. It was editorial policy, or common practice of the contributing group, to include rather lengthy discussions of the published manuscript. This practice continued throughout the first decade of publication.

In contrasting such papers with the type of material published today, one gets the impression that many of the early contributions were deliberately chosen for their inspirational and educational value. A large number of "research" papers were in the nature of reviews of specific subjects, and most of them closed with an exhortation to the researcher and clinician to look more carefully and critically into the problems of dental research. Considering the status of dental research at that time, the desirability of such publications can well be recognized. Very few of the practicing dentists had the benefit of basic training in the scientific aspects of their profession and, for example, the 1930 edition of *American Men of Science* listed only five dentists as research scientists. It is encouraging to note that the 1950 edition lists almost 200 names from this Association alone.

In the very first volume of the *Journal* there appears an article in which the authors express this teaching philosophy. Drs. Lederur and Riethmueller in concluding an article devoted principally to case reports on oral tumors said, "It is our hope that this contribution will help to stimulate closer attention, on the part of the general practitioner, to early recognition of neoplasms and that it will discourage haphazard and temporizing attempts at operation, which have caused many a patient to seek surgical aid too late."

Another factor which may have contributed to the difference in the type of research report appearing at present, as compared to thirty years

ago, is the change in research itself. Much of the dental research at that time seems to have been conducted with a definite application in mind, as, for example, the improvement of treatment materials, procedures, and technics. As important as such developmental studies may have been, their results seldom produce the type of basic evidence essential to a full understanding of the underlying biologic problem—namely, the disease itself.

It is true that in addition to the developmental type of research, there was a good deal of work going on three or four decades ago in the study both of the basic structure and properties of normal oral tissues, and of the underlying causes of oral pathologies. Again, when one looks over the reports of these researches, he notes evidence of a specifically planned approach. It seems to me that we had a situation analogous to that found in the related sciences of medicine, chemistry, and bacteriology when a few outstanding workers dominated each field of endeavor. It was an era of concepts and schools of thought, and most of the research effort was directed at proving or disproving current hypotheses. Today, we have more or less broken away from this philosophy, and we concern ourselves with establishing fundamental facts about biologic phenomena. Research planning is no longer predicated on the testing of previously established postulates. It is, instead, motivated by the desire for basic knowledge, which, when amassed in sufficient quantity, will lead plainly and precisely to the solution of the existing problems.

All of this has been most succinctly expressed by Dr. Irving Langmuir, a Nobel Prize winner in chemistry, who said, "Only a small part of scientific progress has resulted from a planned search for specific objectives. A much more important part has been made possible by the freedom of the scientist to follow his own curiosity in search of truth." Many of you who have visited the Dental Institute in Bethesda may have seen this quotation hanging on the wall in Dr. Trendley Dean's office. It has come to be our motto, and we will continue to live by it.

Today, as the horizons in dental research are growing broader, the activities are growing sharper with the realization of the need for highly specialized knowledge in the many different aspects of the oral disease problem. For example, we can no longer debate Miller's chemico-parasitic theory of dental caries as such without unraveling the complex interrelationships of the oral microflora. We no longer consider these organisms from the standpoint of the good or harm they may do to the host, but rather how they are affected by the activities of all other microorganisms in their environment. Again, in the implication of specific organisms in the etiology of dental caries, we must realize that bacteriology as an exact science demands a full knowledge of the components and activities of the entire oral flora as essential in any attempt to demonstrate such a relationship. In considering the etiologic role of a specific organism, attention must also be given to the manner in which all of the other members of the oral environment influence its internal and external activities. We no longer deal only in classical bacteriology, but go further into such matters as the chemical functioning, molecular composition, and physiologic activities of the bacterial cells themselves.

At present, we are not so much concerned whether or not any single bacterium or group of bacteria is responsible for periodontal disease; we have more or less conceded that infection as an etiologic factor is secondary to some noninfective factor. If this be true, it is necessary to learn more about the fundamental processes that govern the activities of living tissue cells. It becomes essential that we know about the processes going on within the

cell itself, and that we determine the physical and chemical basis on which its normal functions depend. Many new technics are appearing for such investigations. Dental research must assimilate and exploit these current developments in biochemistry, histochemistry, enzyme chemistry, and cellular physiology.

During the past decade we have seen notable advances in instrumentation and analytic technics. The newer microchemical methods have been adapted to studies of the components of saliva. We now have the flame photometer for making rapid and highly accurate chemical analyses. The newer methods for amino acid assay of proteins, as adapted for analysis of the organic fraction of the enamel and dentin, offer the opportunity to learn much more about the ultimate composition and properties of those tissues. In addition, this latter approach as applied to other problems should in the near future extend our knowledge regarding the importance of dietary factors beyond the carbohydrates, minerals, and vitamins.

As we look to the future we can predict that in order to understand and explain even the most common of dental diseases, dental caries, it is of utmost importance that we develop a much more detailed knowledge of the structure of the tooth itself. After nearly a century of microscopic investigation, there is still not a clear picture of the crystalline or the organic components of the enamel and dentin. With the advent of specialized tools, such as the electron microscope, rapid strides should be made toward a better understanding of this problem.

There can be little doubt that radioisotopes will play an increasingly important role among the tools now available to the investigator in dental science. Already researchers using these tools have contributed significant advances to our knowledge. For example, one notes the studies concerned with enamel permeability, calcification, and mineral exchange. Fortunately, some of the isotopes which lend themselves most readily to application in biologic investigation are among those of greatest interest to the dental research worker.

Although research in the physical sciences has proceeded at a faster rate than has research in oral diseases, we are now in a position to apply newer methodology in our field of endeavor and should make every effort to do so.

In summary, I can do no better than repeat the words of Dr. Vannevar Bush from one of his recent publications: "All this gives zest to our endeavor. It makes the study of man by man the most challenging problem that man can conceive. There will be many researchers, many papers, many lives lived, before there can emerge that integrated, complete understanding which alone can form a sound and adequate scientific base for the profession of medicine. And in the meantime we pursue individual paths, which continually diverge, which become daily more specialized; and there is danger that the pioneers on the boundary of knowledge will completely lose touch with one another, that we shall learn more and more about less and less, and that the grand design may be completely obscured by the inconsequential detail which we pile up before it."

Personally, I can fully agree with Dr. Bush when he says he has no solution for the quandary in which we find ourselves. I do believe, however, that the primary objective of organizations such as ours should be to meet the problems resulting from this specialization. It is for the very purpose of communication and education that we have just been assembled in this meeting of our International Association, where personal contacts among scientists are afforded. In addition, our publications serve to disseminate the latest

scientific findings. These two methods have been the traditional ways for scientific interchange, they have proved to be the most effective and have stood the test of time, and I do believe we can profitably improve the international aspects of our Association's activities in this area, and I plan to work toward this goal.

In conclusion, I wish to express again my appreciation for the honor of being named President of this Association. I shall need the help of every member to make this a successful year.

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IV. Abstracts of Papers Read in General Meeting Sessions

1. Lingual Lesions in Experimental Niacin and Riboflavin Deficiencies in Dogs. *Dimitri A. Afonsky, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* The study was conducted on twelve dogs between 1 and 3 years of age divided into four groups: Group 1, controls receiving food ad libitum; Group 2, paired feeding group; Group 3, niacin deficiency; and Group 4, riboflavin deficiency. The dogs were subjected to extensive examination prior to being put on the experimental diet composed of: basal diet—casein 18 per cent, sucrose 64 per cent, vegetable oil 10 per cent, salt mixture XIV 4 per cent, bone ash 4 per cent; supplements (milligrams per kilogram of body weight per day)—thiamin 0.05, riboflavin 0.1, niacin 0.5, Ca pantothenate 0.22, pyridoxine 0.04, choline 0.2, inositol 0.5, p-aminobenzoic acid 0.5; vitamin A 200 I.U., vitamin D 10 I.U., vitamin E 1.0 mg., and vitamin K 1.0 mg. The supplements were administered daily

by mouth. Observations included examination of the oral cavity, biopsy, blood examination, gastric analysis, and lesions elsewhere. Acute symptoms of the deficiencies were induced several times in individual dogs over a period exceeding 300 days. Niacin deficiency resulted in extensive atrophy of the lingual papillae, anemia, and severe poikilocytosis. The filiform papillae were the first to show atrophy being followed by similar changes in the fungiform papillae until the surface became covered by a thin and smooth epithelium. Atrophy also involved the muscle tissue. Riboflavin deficiency was accomplished by patchy atrophy of the lingual papillae, anemia, blepharitis, corneal opacity, and serotal dermatitis. Histologically the lingual lesion appeared similar to those observed in niacin deficiency. The lesions responded only to niacin and riboflavin administration, respectively, with complete regeneration of the papillary layer. Gastric acidity remained within normal limits in all the experimental dogs.

2. Preparation and Sectioning of Carious Enamel and Dentin for Electron Microscopy, *John T. Albright and David B. Scott, National Institute of Dental Research, National Institutes of Health, Public Health Service, Bethesda, Md.* Thin sections for electron microscopy have been cut from plastic-embedded carious enamel and dentin on a rotary microtome, using the thermal expansion method and glass knives. Suitable small pieces of tissue for embedding have been dissected under a microscope from whole teeth and from transverse or longitudinal tooth slices 1 mm. thick. It has been possible to isolate enamel and dentin which is soft enough to section without demineralization. Harder material has been demineralized with acids previously employed for normal tissue (trichloroacetic, phosphoric, and formic), and with lactic and pyruvic acids, and ethylenediamine tetraacetic acid. Low-power photomicrographs are presented describing the type of observations made during specimen preparation prior to embedding.

As an aid in interpretation of the electron micrographs, the general appearance of sectioned bacteria, and the effects of fixation, acid-treatment and plastic processing have been studied. Single colonies of lactobacilli and streptococci, isolated from agar surfaces, have been subjected to handling similar to that used for carious enamel and dentin, and sections have been examined under the electron microscope. In addition, the general morphology of apatite crystals has been observed in sections made from plastic blocks in which synthetic hydroxyl apatite powder of very small particle size was embedded.

3. Dentistry and Mephenesin in Cerebral Palsy, *Manuel M. Album, Philadelphia Society for Crippled Children and Adults, Philadelphia, Pa.* Mephenesin (alpha-beta-dihydroxy-gamma [2 methylphenoxy] propane. Tolserol, Squibb) was administered to 109 cerebral palsy patients to determine its effectiveness as a muscle relaxant during dental treatment. The age of these patients ranged from 2 years to 34 years. The study was conducted for a period of one year and compared with several control groups. A 10 per cent elixir of Mephenesin was the most effective method of administration to these patients. The dose ranged from 0.5 to 2.0 Gm. per 50 pounds of body weight and apparently did not have any harmful effects. Mephenesin in therapeutic limits has no apparent depressant effect on cerebral cortex. Patients remain coherent and cooperative during treatment. Mephenesin does not affect cerebral palsy patients under barbiturates. The medication produces a feeling of relaxation and euphoria within 20 to 25 minutes. The relaxation period lasts for approximately 45 minutes, the last 15 minutes of

which shows the tapering off of the medication. Flow of saliva decreases 9 to 10 minutes after administrations and remains decreased for 15 minutes before returning to normal. Local anesthesia is produced as a transient condition in the throat and mouth following contact with elixir of Mephenesin. Mephenesin relaxation permits cerebral palsy patients to undergo dental treatment as essentially normal patients.

4. The Histopathology of Dental Caries in the Rhesus Monkey. *Sumter S. Arnim, Dental Branch, University of Texas, Houston, Texas.* Studies of partly demineralized serial sections of carious teeth of the Rhesus monkey reveal a bacterial plaque, associated with the lesion, similar to that seen in human beings. The plaque is composed primarily of filamentous forms. No branching is observed. Other organisms, rods, cocci, and bits of food material are sometimes seen on the plaque surface or in its outermost fringes. Changes occur in enamel well ahead of the bacterial mass. Between the first visible change in enamel and the bacterial plaque there is a gradual loss of morphologic characteristics of the tissue. The lesion in enamel is characterized by: (1) Presence of bacterial plaque; (2) a loss of translucency; (3) microscopic cavitation; (4) changes in color which are related to type of histologic stains, as well as to influx of foreign substances from the mouth into the microscopic porosities; and (5) gross cavitation. There is typical spread of the lesion at the dentinoenamel junction with invasion of dentin. There is evidence of change in dentin well ahead of the advance of microorganisms. No direct association is observed between organic elements of enamel and dentin and the advance of the carious process. In those lesions where lamellae are present they appear to act as a leading pathway for passage of the carious process through enamel. When lamellae are absent, the carious process seems to find no difficulty in attacking this tissue. Cracks in enamel are seen in some specimens, filled with bacteria, without evidence of carious change.

5. A Correlation of the Morphologic and Cytologic Principles Involved in the Development of the Teeth of the Domestic Pig. *James K. Avery, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* A serial section study of tooth development, from the initiation of the dental lamina to the eruption of the teeth, has been carried out. Camera lucida studies and three-dimensional reconstructions have been made. The cytologic activity of the formative cells has been correlated with the changing morphologic picture. Three-dimensional drawings based on the changing morphology and cytology have been made to illustrate the important phases of continuous tooth development. These are the bud stage; early, mid-, and late-cap stage; bell stage; beginning dentinogenesis; beginning amelogenesis; early, mid-, and late-enamel matrix formation; enamel matrix maturation; and tooth eruption.

6. The Amino Acid Content of Human Dentin. *Gino C. Battistone and George W. Burnett, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* The amino acid content of human dentin has been determined by qualitative and quantitative two-dimensional paper chromatography. Dentin for analysis was obtained from permanent, caries-free teeth ground to +60, −100 mesh size, and separated from enamel and cementum by flotation methods. This tissue then was prepared for analysis by decalcifying in 25 per cent disodium versenate (pH 7.0), after which it was washed and dried to a constant weight. A gelatin-like substance was

obtained by boiling undecalcified dentin in distilled water and precipitating with absolute alcohol. Portions of the versene-decalcified dentin and the dentinal extract were hydrolyzed in sealed bombs with 20 per cent HCl at 115° C. for varying periods of time and then oxidized with H_2O_2. Other portions were hydrolyzed in 5 N NaOH for varying periods of time. Qualitative and quantitative chromatographic analysis of the various hydrolysates indicated the percentage composition of the amino acids of dentin to be as follows: arginine, 8.5; histidine, 0.5; lysine, 2.5; aspartic acid, 7.0; alanine, 6.8; glutamic acid, 10.6; glycine, 17.4; hydroxyproline, 16.2; proline, 5.2; serine, 2.7; threonine, 2.2; valine, 2.5; cystine, 0.1; methionine, 0.6; tyrosine, 1.2; tryptophane, 0.3; phenylalanine, leucine, and isoleucine (determined quantitatively as a group), 9.4. The ash content of versene-decalcified dentin was 2.04 per cent, while that of the gelatin-like extract was only 1.64 per cent. The amino acid content of versene-decalcified dentin and water-extracted dentin was quite similar, except that tryptophane was absent from the latter.

7. Stripping Emulsion Radioautography of I-131 Uptake in Human Teeth (In Vitro). *Herbert J. Bartelstone and Henry Wolff, National Institute of Dental Research, National Institutes of Health, U. S. Public Health Service Hospital, Staten Island, N. Y.* Survey radioautographic technics, employed in earlier I-131 studies of teeth, involved separation of section and emulsion, making interpretation of distribution pattern difficult. Three factors now make it possible to keep the emulsion and section in permanent contact throughout processing resulting in exact register of radioautograph with section: (1) 50 micra ground sections can now be made; (2) availability of Kodak Limited Autoradiographic Plates; (3) application of "stripping emulsion" technic of Pele to ground tooth section. Essentially, the technic consists of stripping the 5 micra, uniformly thick, NTB emulsion from its plate and floating it on the surface of section mounted on chrome-alum-gelatin subbed slide. After processing, the permanently mounted section and emulsion can be studied microscopically. Photomicrographs of superimposed section and radioautograph of human teeth, enamel of which was exposed to I-131 solution, indicate: (1) Diffuse uptake in sound enamel, (2) increased uptake in poorly calcified and carious enamel and in area of enamel lamellae, (3) increased uptake in dentin at end of lamellae, (4) uptake can be correlated with cut end of single dentinal tubule, (5) increased uptake in interglobular and peripheral root dentin, and (6) uptake in cementum.

8. The Structure of the Epithelial Attachment Studied by Phase Contrast Microscopy. *Louis J. Baume, The George Williams Hooper Foundation and the Division of Dental Medicine, College of Dentistry, University of California, San Francisco, Calif.* The gingiva attached to the crowns of erupting permanent incisors and first molars of autopsy specimens of Rhesus monkeys, which had been preserved in 10 per cent neutral formalin solution for periods varying from one week up to two years, as well as of human beings obtained at biopsy, have been studied. The firmness of the epithelial attachment to the enamel surfaces was found to be dependent upon the duration of fixation, being greatest in the living condition and being absent in extensively fixed specimens. Under the phase contrast microscope the latter exhibited little structural detail and the film scratched off the attaching enamel surface showed an almost homogenous cuticular-like aspect. Fresh, unfixed, and unstained gingival epithelium, conversely displayed prominent tonofibular structures which were continuous throughout the epithelial sheet. At the epithelio-enamel junction they were seen to extend into a basement membrane

and the reticulum of the adjacent lamina propria. The tonofibrils showed different arrangement and behavior in various types of epithelium. In oral surface epithelium they were arranged in bundles of 15 to 25 units running to the side of the nucleus from one cell into another, forming, with their slightly enlarged intercellular portions, the intercellular bridges; they disappeared in the surface layer, which consequently was shed. Enamel epithelium possessed only a few coarse tonofibrils which accepted some fixation. When an erupting crown was denuded of its attached epithelium, the remaining film on the tooth surface in the fresh condition distinctly showed under the phase contrast microscope the broken off ends of the tonofibrils. It was possible to demonstrate their actual continuity with the fibril network of the dentin at the tooth neck. The sum of these observations suggests that both oral and enamel epithelium are attached to the tooth by means of tonofibrils. Their existence is bound to the life of the supporting cells, and therefore is difficult to reveal with ordinary histologic methods.

9. Effect of Tryptophane Deficiency Upon the Jaws of Rats. *L. Baretta, S. Bernick, E. Geiger, and Wm. Bergren, School of Dentistry and School of Medicine, University of Southern California, Los Angeles, Calif.* Albino rats of the Southern California strain at 4 weeks of age were divided into three groups. The experimental group was placed on a tryptophane-deficient diet for three weeks and then repleted up to a total of seven weeks. The other two groups served as positive and negative controls. Both experimental and control animals were sacrificed at the end of three weeks and seven weeks, and histologic studies of the mandibular condyles and the periodontium of the molar teeth of both jaws were made. In the experimental animals the mandibular condyles showed a decrease in the width of the cartilage, varying from a narrowing at three weeks to an extreme thinning at seven weeks. The contents of the marrow spaces were transformed from myeloid elements to fatty tissue, and the trabeculae became thinner as the deficiency continued. The trabeculae of the interdental and interradicular bones were found to be thinner and scantier (osteoporosis) in the deficient animals.

10. Electron Microscopy of Carious Dentin. *Sol Bernick and Richard Baker, School of Dentistry and School of Medicine, University of Southern California, Los Angeles, Calif.* Microscopic structure of carious dentin has been studied by the use of ultrathin sections with the electron microscope. Micrographs are presented showing the different zone of degeneration based on the degree of bacterial invasion. The first zone is characterized by complete decalcification and decomposition of the dentin. An area of incipient decalcification in which the bacteria have invaded the tubules is the second zone. In this region sclerosed tubules whose lumina have become obliterated are found. Last, a layer far from the area of decalcification shows a few tubules invaded by microorganisms.

11. Caries-Like Enamel Changes by Chemical Means. *F. C. Besic, The Walter G. Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* Typical macroscopic characteristics of true caries of human enamel appear to have been duplicated in vitro by the action of a number of acid solutions containing calcium and phosphate ions in less than maximal concentration. The same acid solutions with low concentrations of calcium and phosphate ions produce on enamel surfaces changes which do not resemble natural caries. The same acid solutions with maximal calcium and phosphate ion

concentration (in some cases at pH 3.58) appear to produce no effect on enamel in vitro. Portions of gross enamel specimens were exposed for various periods of time to hydrochloric, acetic, lactic, and carbonic acid solutions containing calcium and phosphate ions in several different concentrations to produce the various effects just mentioned.

12. Histochemical Studies of the Calcifying Tooth. I. *G. Bevelander and Percy L. Johnson, Department of Histology, New York University College of Dentistry, New York, N. Y.* Examination of developing teeth treated to show the localization of nucleic acids reveals that there are small amounts of both DNRA and RNA in the cells of the stellate reticulum and in the cells of the dental pulp. Examination of the odontoblasts shows that RNA appears in the cytoplasm of these cells in the early stages of differentiation and continues to increase appreciably to a maximum amount during cellular activity. The ameloblasts likewise gradually acquire considerable amounts of RNA in the form of rods which appear to be localized in the supra- and infranuclear positions of the cells. Sections of the developing tooth stained by the Hotchkiss method for mucopolysaccharides show that the components of the stellate reticulum are Hotchkiss-positive as are the ameloblasts, the dentinal enamel membrane, Korff's fibers, odontoblasts, and the matrix and cells of the dental pulp. As calcification of the tooth takes place, the predentin acquires a mild affinity for the stain and subsequently increases in intensity as calcification proceeds. The enamel shows little or no reaction to the Hotchkiss stain procedure. In localizing acid polysaccharides in the developing tooth, it was observed that the matrix of the stellate reticulum as well as the matrix of the dental pulp give strong metachromatic reactions. Neither the ameloblasts nor the enamel reveal the presence of acid polysaccharides. In our preparations predentin is intensely metachromatic. With the advent of calcification the metachromasia of the calcifying dentin is gradually replaced by an orthochromatic reaction. In still more advanced stages of calcification, dentin loses both its metachromatic and orthochromatic properties.

13. Growth Pattern and Role of the Condylar and Angular Cartilages in the Morphogenesis of the Mandible. *S. N. Bhaskar, Department of Oral Pathology, University of Illinois College of Dentistry, Chicago, Ill.* A serial histologic study on eighty-four rats varying from 13 days insemination age to 30 days of life was undertaken to determine the directions and rates of growth of the condylar and angular cartilages and their role in the mandibular growth. At 17 days insemination age the condylar and angular cartilages differentiated from a common cartilaginous plate in the region of the mandibular ossification center. From this area a superior focus of chondrocytes grew backward and slightly outward and upward as the condylar cartilage, while an inferior focus grew backward and slightly downward and outward as the angular cartilage. These directions of growth prevailed up to about five days after birth, when the growth of the condylar cartilage changed suddenly to a predominantly upward and a slightly outward and backward direction. Prior to this change in the direction of condylar growth, the mandible grew primarily in length; after the change in directional growth the mandible shifted progressively caudad. Quantitative measurements showed that the change in direction of condylar growth coincided with the increase in the height of tooth germs and the alveolar process. Both the condylar and angular cartilages showed a prenatal phase of active growth, a neonatal arrest phase, and finally another phase of rapid growth. Measurements revealed that the anteroposterior length of the mandible, the height of its alveolar process, and the height of the tooth germs coincided with growth rates of the condylar cartilage.

14. Changes of Enamel Surface Related to Age. *Baldev Raj Bhussry and Basil G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* To correlate possible variations in color of younger and older teeth with caries, extracted teeth were divided into five groups, with ten years' difference in posteruptive age. All the available history of these teeth was recorded. Ground sections were prepared at different levels to facilitate a comparison of all the enamel surfaces. Gingival, middle, and occlusal thirds were examined separately and a quantitative measurement of the extent of surface pigmentation was tabulated. Analysis of the data shows the tendency of an increase in the amount and intensity of surface pigmentation with increasing age. Decalcification of some of these sections under the microscope indicates that the pigmented areas are more resistant to decalcification than the rest of the enamel.

15. The Non-Critical Character of Some Standard Cephalometric Norms. *William Biederman, New York University, College of Dentistry, New York, N. Y.* This study is an analysis of pre- and postoperative cephalometric records of seven successful surgical reductions of mandibular prognathism. Five of these cases were done jointly by the New York University Orthodontics and Oral Surgery Departments and two cases were described by Downs. The facial esthetics and occlusion in all seven cases were markedly improved, and therefore were used to test the essential reliability of using the norms in cases such as these. The use of such norms for diagnosis or prognosis implies (a) that malocclusions would present marked deviations from the range of normal, and (b) that successful treatment of them would result at least in diminishing the extent of such deviations. Our findings do not bear out these implications. Some values are descriptive, but not critical. Others not only are not critical, but are actually misleading, because the deviations from normal are increased despite the dramatically successful results. Particularly noncritical and unreliable are the mandibular plane angle and the incisor-mandibular plane angle, the two values used by Tweed as criteria for diagnosis and treatment. Photographs, roentgenograms, graphs, and tables are used to illustrate the findings and conclusions. New methods for determining occlusal bite and jet are described and illustrated.

16. The Amino Acid Content of Human Salivary Mucin, *R. Q. Blackwell and L. S. Fosdick, Northwestern University School of Dentistry, Chicago, Ill.* Amino acids in human salivary mucin were determined by use of the ion exchange column technic of Moore and Stein and by paper chromatography. The mucin was precipitated from 14 L. of pooled human saliva by pouring the saliva as collected into 6 per cent acetic acid. The saliva-acetic acid mixture had a final pH of 3. The precipitated mucin was separated by centrifuging and was dried under a vacuum while frozen to yield 25.1 grams of the crude mucin. Ten grams of this material was suspended in approximately 1 L. of HCl solution at pH 2 and 50 mg. of purified pepsin added to digest other proteins. The suspension was allowed to stand several days at 0° to 3° C., and one day at room temperature, to complete the digestion of other proteins. The pH was adjusted to 3 with NaOH, and the mucin was separated by centrifuging. It was suspended in a small amount of water, placed in a cellophane bag, and dialyzed at 0° to 3° C. for several days against distilled water (several changes of 2 L. each). After dialysis the mucin again was lyophilized and 1 Gm. of the dry powder was hydrolyzed with 6 N HCl for forty-eight hours. The hydrolysate was studied by two-dimensional paper chromatography and ion-exchange column chromatography. The amino acid content was found to be similar to that of gastric mucin.

17. Determination of Copper and Lead in Surface and Subsurface Enamel. *Finn Brudevold, Eastman Dental Dispensary, and Luville T. Steadman, University of Rochester, Rochester, N. Y.* Spectrographic determinations of copper and lead and chemical determinations of phosphorus were made of successive acid etchings from the enamel surface of different teeth. The copper phosphorus ratio ranged from 0 to 0.0004, with the copper content sometimes greater and sometimes less in the surface posteruptively, suggesting that copper is not picked up by the enamel surface. This was further substantiated by the observation that the copper content was not increased in teeth which had been exposed to copper solutions. The lead content, which was consistently greater in the surface than in the subsurface enamel, was found to be approximately ten times greater than that of copper in untreated teeth. Contrary to copper, lead was found to be readily taken up by the enamel surface, the uptake generally increasing with exposure to greater concentrations. A few observations suggest that tin is taken up by the enamel surface in the same way as lead. Although it was shown that lead was taken up by the enamel surface, no appreciable reduction was detected in the surface solubility of enamel which had been exposed to lead solutions.

18. The Effect of Carbohydrates and Inhibitors on the Respiration Rate of Human Saliva. *George W. Burnett, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* The relationship of the oxygen consumption of whole, stimulated human saliva to the addition of mono-, di-, and polysaccharides and inhibitors has been investigated by manometric methods. The average basic oxygen consumption of the salivas of forty-four individuals varied between 1.14 and 4.70 μl. per minute. The basic oxygen consumption of cannulated parotid saliva was very low, while that of whole saliva was inhibited by Seitz-filtering, high speed centrifuging, boiling, and treatment with cyanide or strong mineral acid. The addition of 2 per cent glucose to whole saliva of ten individuals gave results ranging from no stimulation of oxygen consumption to an increase of 66.1 per cent over normal, with a mean increase of 28.1 per cent. The addition of 2 per cent starch to such salivas gave results ranging from no stimulation of oxygen consumption to a maximum increase of 38.6 per cent, with a mean increase of 16.2 per cent. The addition of 1 to 5 ppm NaF to whole salivas did not decrease their oxygen consumption significantly, but the addition of 200 ppm caused an average decrease of 6 per cent. The mean percentage increase in oxygen consumption of these salivas plus 2 per cent glucose was 25 per cent; the increase for the salivas plus 2 per cent glucose plus 200 ppm NaF was 19 per cent; the increase for the salivas plus 2 per cent starch plus 200 ppm NaF was 12.6 per cent. Concentrations of sodium fluoride, lead fluoride, calcium monofluorophosphate, and potassium monofluorophosphate ranging from 1.0 to 1.4 per cent effectively inhibited oxygen consumption of whole saliva, while similar concentrations of calcium fluoride did not do so.

19. A Histochemical Study of Salivary Glands of the Tongue. *M. S. Burstone, Department of Anatomy, University of Chicago, Chicago, Ill.* Certain histochemical methods were used in order to demonstrate and distinguish between the intracellular precursors of mucous and serous gland secretions. The methods used may also give some indication of the submicroscopic organization of the secretory substances which is reflected in the physical properties of saliva. The tongues of fifteen young adult C57 mice were fixed by freezing in liquid nitrogen and dehydrated in a vacuum. Sections were

stained using hematoxylin and eosin, mucicarmine, toluidine blue, methyl green-pyronin, and the periodic acid-leucofuchsin method. Prior to staining with the periodic acid-leucofuchsin method some sections were treated with various enzymes (amylase, trypsin, collagenase) in order to further characterize the intracellular precursors of mucous and serous secretions. The cytoplasm of mucous cells stains metachromatically with toluidine blue; green with methyl green-pyronin; and is intensely periodic acid-leucofuchsin positive. The cytoplasm of serous cells stains orthochromatically with toluidine blue; pink with methyl green-pyronin; and is periodic acid-leucofuchsin to a lesser degree than observed in the mucous cells.

20. The Attachment and Reattachment of the Enamel Epithelium in the Incisors of Monkeys. *Earl O. Butcher, Department of Anatomy, College of Dentistry, New York University, New York, N. Y.* Monkey jaws were fixed in formalin and the lingual portion of the dentin of the incisors was burred away, leaving the gingiva and the labial portion of the tooth to be decalcified in 5 per cent trichloroacetic acid for seventy-two hours and in 5 per cent formic acid for forty-eight hours. Following paraffin sectioning, much of the enamel and gingiva retained their relations. In the monkey incisors, prolongations of the primary cuticle penetrate the enamel. The enamel epithelium adheres securely to the cuticle, for when it is pulled away layers of cells remain attached to the cuticle. The depth of the gingival sulcus varies and the enamel epithelium adjacent to the enamel and distal to the bottom of the sulcus keratinizes, which provides additional support in the attachment. To study the reattachment, incisors were extracted from monkeys under anesthesia. All organic material was carefully burred away from the labial side and the pulp was removed via the apex. In other instances, extracted incisors with pulps removed were placed in 10 per cent formalin for ten minutes and then washed in water. Following either treatment, the incisors were replaced in their alveoli. The gingiva was examined at intervals and the teeth became firmly attached. The monkeys were sacrificed one to nine months after the extractions and the teeth were histologically prepared. The enamel epithelium readhered to the primary cuticle. An intraepithelial split developed, forming a sulcus as in normal teeth.

21. A Study of the Relation Between Dental Department Personnel and Their Patients in the Transmission of Upper Respiratory Diseases. *W. J. Carter and J. R. Seal USN Naval Medical Research Unit No. 4, Great Lakes, Ill.* Recent studies by the Naval Medical Research Unit No. 4 revealed high incidence of upper respiratory infections among recruit populations aboard the Naval Training Center during winter months. These infections, at times involving 80 per cent of the recruit personnel, were consistently higher than rates experienced by nonrecruit populations. The attack rates in recruits were considered in terms of risk to dental officers and assistants who treated these men as dental patients. Comparisons were made of all upper respiratory infections experienced by dental teams working on new recruits, recruits in training, and nonrecruits. Data were collected by means of a weekly questionnaire and periodic throat cultures from each dental officer and his assistant and their respective patients. The results indicate that dental teams working on new recruits have the most frequent infections of the three groups. Dental personnel working on recruits do not experience upper respiratory infections commensurate with the number of infections seen in their patients, even during periods when epidemics of colds and sore

throats were prevalent among recruits. The presence of hemolytic streptococci, while numerous in recruits' throats, was almost negligible in the throats of dental personnel. There was no instance in which spread of a typable strain of hemolytic streptococci could be demonstrated between a patient and a member of the dental team.

22. Determination of the Enzyme Content of Whole Saliva. *Howard H. Chauncey and Vincent F. Lisanti, Tufts College Dental School, Boston, Mass.* The presence of ten enzymes in whole saliva was established. The enzymes measured were acid phosphatase, alkaline phosphatase, total esterase, cholinesterase, lipase, beta-D-galactosidase, beta-glucuronidase, sulfatase, lysozyme, and hyaluronidase. Paraffin-stimulated saliva samples were taken from fifty-six persons, and the range of activity and mean activity of these enzymes were determined. Cannulated parotid saliva was obtained from a sample of the group and tested for the presence of these enzymes; mean and range of activity were determined. Inoculums of whole saliva were incubated in brain-heart infusion broth to determine whether the oral flora was capable of elaborating these enzymes. Parotid saliva was found to contain acid phosphatase, esterases, cholinesterase, lipase, beta-glucuronidase, and lysozyme. The parotid secretion contained 1 to 60 per cent of the activity found in whole saliva with the exception of lysozyme which was greater than that of whole saliva. The microorganisms of the oral cavity were found to produce all the enzymes except lysozyme. The subjects in this study were classified by means of clinical and radiographic procedures into five groups according to the condition of the oral cavity. Since variations in enzyme activity were noted, correlations were done to determine the significance of these variations.

23. Estimation of Sample Size in Experiments. *Neal W. Chilton and John W. Fertig, School of Public Health of the Faculty of Medicine, Columbia University, New York, N. Y.* The estimation of sample size in experiments necessary for statistical significance to be asserted depends upon the computed constants to be compared (averages or proportions), and idea of how the data are going to run, what difference between samples is expected, and the significance level and power of the test selected. Once these have been determined, either through preliminary experimentation or prior knowledge of the variables under study, the sizes of the samples can be estimated. Formulas and examples from the dental literature are presented which will enable the research worker to estimate the sizes of such samples for different significance levels and powers. In addition, tables for rapid estimation of sample size have been constructed.

24. Reduction in Caries in Experimental Animals by Pinus Strobus Substance. *Thomas B. Coolidge and Rebecca Woodson, Zoller Memorial Dental Clinic and Department of Biochemistry, University of Chicago, Chicago, Ill.* A substance present in the wood of *pinus strobus* has been isolated and crystallized. This substance inhibits the growth of homofermentative lactobacilli in low concentrations and is presumably responsible for a reduction in caries production in certain experimental animals fed cariogenic diets, when the animals have had access to white pine shavings.

25. The Use of a Frequency Counter for Establishing Relationships Between Action Potentials and Contraction Forces in the Masseter

Muscle. *W. A. Curby, Tufts College Dental School, Boston, Mass.* In an attempt to correlate the action potentials of the masseter muscle with actual biting forces, a new method of evaluation was devised. Recordings of the electrical activity of the masseter were amplified and then recorded on an electrical impulse counter. The counter was designed in such a way that it counted only spikes whose amplitude was greater than a value controlled by the observer. Force measurements were made with a special resistance strain gauge. Simultaneous force and electrical activity measurements were taken in periods of ten seconds' duration. A total of nine subjects was evaluated in the study. It was found that for given cut-off levels of the muscle tested, a linear correlation existed between muscle action potentials and contraction forces.

26. Endodontic Evidence Revealing Abstruse Aspects in the Etiology of Dental Caries. *Victor H. Dietz, St. Louis, Mo.* Thirty freshly extracted normal anterior teeth were placed within the peritoneal cavity of a guinea pig. Teeth were removed at approximately five-day intervals for a duration of 100 days. In each of ten animals two teeth were inserted, one "old" and one "young," in pairs. Teeth removed after fifty days exhibited smooth, opaque-white areas in the enamel surface. These lesions were determined as resulting from proteolysis. Young teeth quite consistently evidenced larger developed lesions. These findings correlate with clinical, radiographic, and endodontic evidence. The latter material was obtained from ninety-three patients who were selected from 1017 patients. The mechanism of dental caries appears to be a diphasic oscillatory phenomenon—diphasic because it is both proteolytic and glycolytic, and oscillatory by the apparent mechanism of how these distinct hydrolyses occur simultaneously. In this diphasic oscillatory theory, three distinct posteruptive, nontraumatic, acquired lesions of the enamel are recognized: (1) decalcific lesions, (2) erosive lesions, and (3) carious lesions. Dental caries appears to be the result of both decalcific mechanism (the chemicoparasitic theory of Miller) and erosive mechanism by a diffusible proteolytic enzyme elaborated by the vital dental pulp. Diffusion experiments with Pyrozone, Superoxol, hyaluronic acid, and hyaluronidase demonstrate that the enamel is more permeable in younger teeth than old. Hyperexosmosis, occurring under the bacterial plaque, draws the proteolytic moiety to the enamel surface. The heliotropic phenomenon in the sites of occurrence of the various lesions (decalcific, erosive, and carious) appears wholly explainable on the basis of diffusion, vital propulsion of dentinal fluid, and surface tension phenomena.

27. The Influence of Latitude and Distance From Seacoast on Dental Disease. *James M. Dunning, Harvard School of Dental Medicine, Boston, Mass.* A number of reports have been made upon the geographic variations in dental disease found within the United States, and some reports are available on geographic variations among relatively homogeneous population groups elsewhere in the world. This study brings together these reports, plots them on maps, and applies statistical analysis to the plots. A convincing pattern emerges for the United States, showing dental disease, chiefly caries, to be correlated with latitude ($r = 0.844$ along the Atlantic Coast and 0.923 on the one hundredth meridian of west longitude) and with distance from seacoast ($r = 0.847$ along the forty-third parallel of north latitude). These coefficients are statistically significant. Similar patterns are found in South Africa ($r = 0.398$ for distance from seacoast) and Australia ($r = 0.540$ for latitude). No geographic pattern is found in the New Zealand data. An analysis of

factors which may be linked with variations in dental disease shows mean annual hours of sunshine and relative humidity to present very similar patterns. Temperature is related to latitude and to demonstrated variations in diet. Data from the U. S. Department of Agriculture are presented which show much greater consumption of baked goods (refined carbohydrates) in the north. Urbanization, thought to be related to latitude, does not relate significantly to caries as shown in an analysis of forty-one United States cities. Fluoride content of water supplies and rainfall and humidity variations may help explain differences in dental disease with distance from seacoast.

28. The Transplacental Transmission of Antibody to the Virus of Herpes Simplex. *S. A. Ellison, R. Moses, and H. M. Rose, Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, N. Y.* Samples of blood from twenty-seven mothers and their infants were obtained at the time of delivery. These were examined for their content of antibody to the virus of herpes simplex by an in ovo neutralization test and a quantitative complement fixation test. The latter procedure will be described since it is essentially a new method in which the antigen is prepared by fractionating chorio-allantoic membranes of infected chick embryos in an electrophoresis-convection apparatus. The results clearly demonstrate that antibody to herpes simplex virus is regularly transmitted across the placenta in significant amounts. The neutralization titer of the serums of 22 of the infants was within a twofold dilution of that of the maternal serum; four were higher; one was lower. The complement fixation titer of the serums of 16 of the infants was within a twofold dilution of the maternal titer; eight were lower; two were higher. These results will be discussed.

29. Binding of Calcium in Connective Tissue. *Milton B. Engel, Norman R. Joseph, and Hubert R. Catchpole, University of Illinois, Colleges of Dentistry, Pharmacy, and Medicine, Chicago, Ill.* The density of colloidal charge, x, in equivalents of base-binding anionic groups was estimated in the following tissues: monkey dentin, sternum, and gingiva; rabbit epiphysis and skin. Liquid junction potentials were measured at boundaries in the tissues. Base line potentials with 0.15 M NaCl were determined, followed by observations of the displaced potentials obtained with 0.015 M NaCl. Colloid charge, x, was then estimated from the formula $E_d = -12.3 + 215x$ where E_d is the displaced potential in millivolts. Following restoration of the base line value, the tissue was equilibrated with 0.15 M NaCl plus 0.01 M $CaCl_2$. The modified colloid charge density, x′, was determined by measurement of a second dilution potential, $E_{d'}$, also observed with 0.015 M NaCl. Bound calcium was determined by the difference (x-x′). From the modified charge density, x′, the Donnan ratio r was estimated, leading to values of ionic Na and ionic Ca in the calcified connective tissues. Total calcium was estimated as the sum of ionic and bound calcium. The results have been expressed in terms of a nomogram with the following variables: x, x′, bound Ca, ionic Ca, total Ca, ionic Na, and applied calcium ions in isotonic saline. An equilibrium constant has been calculated for the reaction $Ca^{++} + x^{-} = CaX$. Administration of parathyroid extract reduces the density of charge, x, and therefore the bound Ca, in all of these tissues. Figures for Ca, Na, and Cl determined electrometrically agree well with analytic figures for both dense and loose tissues.

30. Salivary Contribution to Enamel Maturation and Caries Resistance. *Robert J. Fanning, James H. Shaw, and Reidar F. Sognnaes, Harvard School of Dental Medicine, Boston, Mass.* The major salivary glands were

surgically removed in five groups of twelve white rats each at 21, 31, 41, 51, and 61 days of age, respectively. During this initial postweaning period of forty days, all rats were maintained on a noncariogenic adequate purified diet, containing a ratio of 17 Gm. fat, 64 Gm. protein, 4 Gm. salt mixture, 2 Gm. 1:20 liver concentrate, and 2 Gm. whole liver substance, as well as all required vitamins. Two rats from each group were sacrificed on the sixty-first day of age, at which time no caries had developed. The remaining rats were transferred to a purified caries-producing diet (Shaw, *J. D. Res.* **26**: 47, 1947) for an additional 114 days. The experiment was terminated when all animals were 175 days of age. At that time the number of carious molars, the number and extent of carious lesions were determined by binocular examination. The group of rats desalivated at 21 days of age had the highest incidence of dental caries. A progressive decrease in caries was noted as the length of salivary exposure of the teeth was increased. Statistically, there was a highly significant difference between the extreme groups, desalivated at 21 versus 61 days, respectively. These data suggest that saliva has an essential influence on the teeth in the early posteruptive period which results in a higher resistance to the external caries-producing factors.

31. Effects of Several Fluoride Reagents Upon the Surface Structure of Dental Enamel. *Robert B. Fischer and Joseph C. Muhler, Department of Chemistry, Indiana University, Bloomington, Ind.* Electron diffraction and x-ray diffraction studies have been made of powdered dental enamel following treatments with several different fluoride reagents. Hydrofluoric acid causes a rapid transformation of the apatite structure to one of calcium fluoride, and the change is predominately upon the surface of the enamel. Sodium fluoride causes a similar but less rapid change, with the rate increasing as the pH is lowered. Results observed with potassium fluoride are approximately the same as those observed with sodium fluoride. Sodium monofluorophosphate does not result in a change of apatite structure to calcium fluoride structure during treatments for as long as one week in a 2 per cent solution at a pH as low as 2. Sodium fluosilicate also causes a transformation to calcium fluoride, but the rate is slower than with sodium fluoride, and the changes develop somewhat within the particles rather than just on the surfaces. The effects of stannous fluoride are distinctively different than those of other reagents. The surface apatite structure is replaced by an amorphous structure, even during very brief treatments, and this amorphous substance may be rendered crystalline by heating. The mechanism must involve both the tin and the fluoride, because stannous chloride treatments result in practically no alteration of the original apatite structure. X-ray diffraction studies of whole teeth following treatments with various fluoride reagents indicate that the effects of the reagents upon whole teeth are substantially the same as those upon powdered enamel.

32. Studies on Rat Oral Lactobacilli. *R. J. Fitzgerald, H. V. Jordan, and I. Zipkin, National Institute of Dental Research, Bethesda, Md.* A number of bacteriologic studies on the oral flora of white rats fed various cariogenic and noncariogenic diets have yielded results which indicate a lack of etiological relationship between rat oral lactobacilli and caries. In addition, it appears that oral lactobacillus counts of rats are not a valid index of the severity of the caries attack, first, because the lactobacillus count of weanling rats may actually decrease when these animals are placed on a cariogenic diet and, second, lactobacillus counts of rats maintained on noncariogenic diets frequently may equal or surpass those of animals on cariogenic diets. The types of lactobacilli indigenous to the oral cavity of the white rat differ in

several important biochemical and nutritional characteristics from those found in the human oral cavity. These organisms seem to be previously undescribed and their characteristics will be presented in some detail.

33. Effect of Fluorides on the Tumor S 37 After Transplantation to Selected Locations in Mice and Guinea Pigs. *Harold S. Fleming, Yale University School of Medicine, Department of Pathology, New Haven, Conn.* The tumor known as Sarcoma 37 was transplanted to selected locations in dba mice and guinea pigs. Young adults of both sexes received transplants of this tumor. For mice the transplantation sites were the brains and axillae; for guinea pigs the sites were the anterior eye chambers and the brains. After transplantation each group of animals was divided so that half could receive fluorides, while the remaining half served as controls. NaF was administered either by drinking water or by intraperitoneal injection. Concentrations of 20 ppm (1-50,000) were used for drinking water and concentrations of 1,000 ppm (1-1,000) were used for injections. Administration of fluorides was over selected periods, usually starting directly after transplantation of the tumor. Drinking water with fluorides was given ad lib. A mouse weighing approximately 20 Gm. received 0.05 c.c. per injection, while a 400 to 500 Gm. guinea pig received 0.5 c.c. per injection. Injections usually were given daily. Treated and control animals were sacrificed intermittently. Fluorides, as given in the amounts stated, did not appear injurious to the animals. Animals bearing transplants of this tumor lived longer and lost less weight, when fluorides were administered, than did the controls. Tumor size was grossly larger in untreated animals. Microscopically, tumors from untreated animals were larger and more vascular than those from treated animals. Tumor cells from treated animals showed less tendency to form rosettelike arrangements with polarized nuclei around blood vessels. It was also observed that spaces between tumor cells were consistently more definite and larger when fluorides had been administered. In addition, the ratio of cytoplasm volume to nuclear volume was much less in these instances. Central portions of treated tumors showed more necrosis, while along the peripheral borders fewer instances of telephase were observed, although there were a greater number of figures representing earlier mitotic stages. From these observations it appears that fluoride administration alters growth of the tumor S 37.

34. An Improved Micro Antimony Electrode and pH Determination in the Periodontal Pocket. *B. K. Forscher, A. G. Paulsen, and W. C. Hess, Georgetown University School of Dentistry, Washington, D. C.* Electrodes of pure antimony cast in glass are not suitable for pH determinations in poorly accessible regions because of the poor mechanical strength and brittle nature of antimony. These disadvantages are avoided by preparing an electrode by electroplating antimony onto a No. 5 dental explorer using the method of Bloom. This electrode is insulated with a chemical-resistant nonconducting resin so that only 2 to 3 mm. of the antimony-coated tip is available for reaction with the medium. The pH of the periodontal pockets in a number of patients was investigated with this instrument. Inspection of the data revealed no apparent relationship between the pH and the depth and location of the periodontal pockets.

35. Chemical Studies in Periodontal Disease. XI. The Volatile Amine Content of Saliva. *L. S. Fosdick, R. Q. Blackwell, and W. J. Carter, Northwestern University Dental School, Chicago, Ill.* Piez, on the basis of paper chromatographic studies on fresh and incubated saliva, found that the saliva

of people suffering from periodontosis consistently contained volatile amines. It was thought that this phenomenon might be used for a rapid chemical test for periodontosis. Five cubic centimeters of saliva was collected in a test tube containing 5 c.c. of acidified ethyl alcohol. The mixture then was centrifuged, and 5 c.c. of the clear solution was diluted with 10 c.c. of 1 N sodium hydroxide and distilled into a 25 c.c. volumetric flask. To the distillate was added 0.2 c.c. of 1 per cent ninhydrin and 10 c.c. of pH 5 buffer, and the mixture was warmed in boiling water for twenty minutes to develop the color. The mixture was cooled and diluted to exactly 25 c.c. with a 50/50 propanol/water solution. The transmission was read on a Coleman Spectrophotometer at 570 $\mu\mu$. The readings were compared with a standard curve prepared from methylamine. The volatile amine content varied from 0 to 26 mg. per 100 c.c. If it is assumed that any value between 2 mg. per 100 c.c. is negative, then 85 per cent of the eighty-eight patients with normal gingivae are negative. In twenty-five cases of frank periodontosis the test is positive in 96 per cent, and in ten cases of periodontitis the test is negative in 80 per cent of the cases.

36. Metabolism of the Protein Matrix of Calcified Tissues Under Normal and Fasting Conditions. *Irving B. Fritz and George W. Burnett, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* Since the incorporation of N^{15}-labeled amino acids into tissues is intimately associated with protein metabolism, and is independent of simple ion exchange, N^{15}-glycine was used to study the in vivo protein metabolism of various calcified and noncalcified tissues of Sprague-Dawley rats. Male rats, weighing approximately 200 Gm. were maintained on a 15 per cent casein diet in separate metabolic cages. They were each fed 150 mg. of 32.0 atom per cent excess N^{15}-glycine in six aliquots over a three-day period, after which they were fasted for various intervals before sacrificing. Comparable tissues from different groups of rats were then collected and analyzed for their N^{15} concentration. The tissues of animals which were sacrificed eighteen hours after the last N^{15}-glycine feeding had the following concentrations of N^{15}, expressed as atom per cent excess: incisor dentin, 0.072; molar dentin, 0.056; femur diaphysis, 0.085; femur epiphysis, 0.197; and plasma proteins, 0.307. In contrast, the tissues of animals which were sacrificed 160 hours after the last N^{15}-glycine feeding had the following concentrations of N^{15}, expressed in similar units: incisor dentin, 0.134; molar dentin, 0.040; femur diaphysis, 0.063; femur epiphysis, 0.107; and plasma proteins 0.165. In general, the N^{15} concentration of various tissue proteins decreased as the animals were fasted, resulting from the catabolism of primarily dynamic tissue protein. In marked contrast, the relative increase in concentration of N^{15} of incisor dentin indicates that a high net anabolism of dentinal protein was maintained during fasting, and formation of new matrix was facilitated by the utilization of nitrogen from the general metabolic pool.

37. The Mechanism of Staphylococcal Coagulation and Fibrinolysis. *Earl B. Gerheim, Horace M. Floyd, James Okubo, Robert Morris, and Ray Prins, Division of Research, University of Detroit School of Dentistry, Detroit, Mich.* Pathogenic strains of *Staphylococcus aureus* are capable, in most cases, of both clotting human plasma and lysing the clot. This staphylococcal coagulation is independent of the normal clotting mechanism which depends upon the formation of thrombin. An equation-like summary gives the mechanism of this bacterial coagulation.

1. Prostaphylocoagulase + Cofactor → Staphylocoagulase (Exotoxin from Staphylococcus) (Plasma component) (Thrombinlike)
2. Fibrinogen + Staphylocoagulase → Fibrin

The mechanism of staphylococcal fibrinolysis is analogous to streptococcal fibrinolysis, that is, the activation of an enzyme precursor (profibrinolysin). It might be pointed out that this is the rationale for the use of the streptococcal factor (streptokinase) in such conditions as hemothorax and burns.

38. An Electro-Anatomic Survey of the Gasserian Ganglion. *Joseph A. Gibilisco, University of Alabama School of Dentistry, Birmingham, Ala.* A dual beam cathode ray oscillograph and supplemental electronic equipment have been adapted for determination of the electrical potentials existing in the Gasserian ganglion. Fifteen cats have been used for this study with the ultimate objective of determining the pathways of sensation such as pain, temperature, and touch. A platinum-stimulating electrode is fixed over the various areas of peripheral distribution of the trigeminal nerve. A nichrome wire pickup electrode is inserted within the body of the Gasserian ganglion or one of its three divisions. Nerve impulses from the point of stimulation pass through the nerve to its ganglion and then to the pickup electrode. The potentials from the pickup electrode are amplified by a preamplifier and pass to the cathode ray oscillograph. Responses to the stimulation have been observed from (1) the cornea, (2) the right maxillary and mandibular gingival tissue, and (3) the right maxillary and mandibular dentition. Investigation has been limited to the right Gasserian ganglion. Intensity of stimulus has shown considerable variation in different locations and different tissues. For example, we have observed that the cornea responds to the lowest intensity, while increased intensity is necessary to induce a response from either the dentin or the enamel. The magnitude of the stimulus needed for an enamel response is approximately 20 to 40 volts. These results indicate the feasibility of using electro-anatomic technics for determining the nature of the fibers found in the divisions of the trigeminal nerve.

39. Relative Abilities of Natural and Artificial Dentition Cases in Judging Differences in Sweetness of Solid Food. *Donald B. Giddon, M. E. Dreisbach, Carl Pfaffman, and R. S. Manly, Tufts College Dental School, Boston, Mass.* A test food for sweetness judgments by the differential threshold method was prepared by making cookies with 6, 7, 8, and 9 ounces of sugar in different batches. Each subject was required to give an estimate concerning the relative sweetness of a morsel of a cookie when he chewed it in his usual manner. Fifteen full-denture cases were studied, both with and without a limitation of 10 seconds on the time for judgment. These cases were compared with fourteen natural dentition cases having a similar average age. On the average the natural dentition cases needed less than one-half as much time as the full-denture cases in order to be ready to give an estimate. Under these conditions it appeared that the full-denture cases required 1.2 Gm. difference in sugar content per morsel in order to give correct estimates three out of four times. The natural dentition cases needed only 0.7 Gm. on the average. With the time restriction, natural dentition cases had the same accuracy of judgment as without, but the denture cases needed nearly 5 Gm. of sugar differential. This finding suggests that inadequacy in masticatory ability may hamper a subject's enjoyment of solid food under certain circumstances.

40. The Demonstration of Aldolase Activity in Sonic Extracts of Lactobacillus casei 4646. *Robert E. Gillis, Department of Microbiology, Western Reserve University, Cleveland, Ohio.* We have reported previously on the existence of glycolytic activity in sonic extracts of *Lactobacillus casei* 4646. The present communication deals with the demonstration of activity of the enzyme aldolase in the sonic extracts. Aldolase catalyzes the following reaction:

Hexosediphosphate $\rightleftharpoons$ 3 phosphoglyceraldehyde + dihydroxyacetone phosphate

The method used to assay the glycolytic extract for aldolase activity is a modification of the Sibley and Lehninger method for assay of animal tissue extracts for this enzyme. It involves the production of a chromogen by the addition of 2, 4-dinitrophenylhydrazine to the triose phosphates produced when hexosediphosphate is split by aldolase. Hydrazine is used as a trapping agent and the hydrazones of the triose phosphates are trapped in a 1:1 ratio. Esterified phosphates are removed by incubation with NaOH. The addition of 2, 4-dinitrophenylhydrazine produces 2, 4-dinitrophenylhydrazones which give a characteristic reddish-violet color. A further test for aldolase activity was devised making use of the following reaction:

$$\underset{\text{Hexosediphosphate}}{\overset{(1)}{\rightleftharpoons}}\ 3\ \underset{\text{Dihydroxyacetone phosphate}}{\text{phosphoglyceraldehyde}} \overset{(2)}{\rightleftharpoons}\ 1\ \text{arseno-3-phosphoglyceric acid} + DPNH_2$$

$$\Uparrow \quad \overset{DPN}{ASO_4}$$

In reaction (2) DPN is reduced to $DPNH_2$. The reaction is followed in the Beckman spectrophotometer by reading DPN at 340 mμ. Data obtained using these methods clearly showed the existence of aldolase activity in the sonic extracts.

41. Effect of Pressure on Tooth Material. *R. E. Glegg, C. P. Leblond and Y. Kumamoto, Department of Anatomy, McGill University, Montreal, Canada.* After injection of Ca^{45} and P^{32} into newborn and very young rats, radioautographs of sections of teeth revealed that a radioactive band appears in the dentin due to the deposition of crystals containing these elements. As time elapses after P^{32} injection, the band approaches the dentinoenamel junction and becomes more widespread. These results are attributed to a rearrangement of the crystals of young dentin due to dissolution and redeposition of crystalline material. It is suggested that mechanical effects, particularly pressure and tension effects, are responsible for the instability of the crystals of dentin. While it is generally believed that mechanical effects influence bone or tooth by producing changes in vascularization and by evoking the appearance of osteoclasts (pressure) or osteoblasts (tension), mechanical effects could not possibly influence the core of the dentin in such a manner. Arguments will be presented in support of the view that these observations are explained by mechanical effects exerted directly on the crystals of dentin. In general, pressure would be responsible for the increased solubility of bone or tooth crystals, in accordance with Riecke's principle. Conversely, tension (which in the mathematical analysis of the problem may be considered as a negative pressure) would promote growth of the crystals.

42. The Effect of Systemic Disturbances Upon the Dental Pulp. *Irving Glickman and Gerald Shklar, Department of Oral Pathology, Tufts College Dental School, Boston, Mass.* The dental pulp of developing and formed

teeth was studied in rats and mice subjected to the following systemic disturbances: acute starvation, protein deprivation, hypothyroidism induced by thiouracil, parenterally administered cortisone, alloxan diabetes, and the alarm reaction of Selye. An effort was made to evaluate pulp changes in teeth devoid of carious involvement. In general it was found that the effects of systemic influences were more notably manifest in the continuously erupting incisors than in the pulps of the molar teeth. Comparable pulpal changes were noted in acute starvation, alloxan diabetes, and in animals subjected to repeated injections of cortisone. The pulpal changes consisted of degeneration of the odontoblastic layer with vacuolization of the cytoplasm, pyknosis, and rupture of the cell membrane. In the remainder of the pulp the cells presented a diminution in number and alteration in morphology. The over-all effect was a change from the normal stellate shape to a spherical, somewhat pyknotic cell. The normally homogeneous matrix of the pulpal tissue was altered so that it appeared as an irregularly fibrinoid meshwork enclosing numerous small vacuoles.

43. Tooth Attachment in Dermoid Cysts. *Henry M. Goldman, Eleanor Covel, and David G. Stahl, Dental Department, Beth Israel Hospital, Boston, Mass.* There has been much discussion recently of the presence of plasma cells and lymphocytes in the connective tissue subjacent to the sulcular epithelium of the tooth. One opinion holds that these inflammatory cells are normal constituents of the gingival tissue, while another believes the presence of these cells to be a sign of a subacute inflammation. It was decided to investigate similar areas in teeth not exposed in the oral cavity. Erupted teeth in dermoid cysts were examined, and microscopic study of the gingival tissue disclosed an infiltrate corresponding to that seen in the oral gingiva. The sulcular epithelium was intact, and subjacent to it was an infiltrate composed chiefly of plasma cells and lymphocytes.

44. Comparative Effect of Sodium Fluoride and Sodium Oxalate on Normal and Neoplastic Tissues In Vitro and In Vivo. *Nicholas G. Grand, School of Dentistry, St. Louis University, St. Louis, Mo.* The purpose of this investigation was to study the comparative effect of various fluoride and oxalate concentrations on normal and neoplastic tissues in vitro and in vivo. The tissues used were selected according to their particular function, e.g., mouse spleen for the ameboid cells, mouse salivary gland for terminal cell cluster formations, chick embryo heart for muscle contractions, chick embryo kidney for dye concentration into the proximal tubules, and neoplastic tissues for successful transplantations and transfers. Adenocarcinoma of the mouse mammary gland and an experimentally produced undifferentiated carcinoma of the mouse parotid gland were employed. The tissue fragments (1 to 2 $mm.^3$) were immersed in either sodium fluoride or sodium oxalate isotonic solutions of known concentrations. The volume of the test solutions was always 1 mm. The tissues were removed at the end of one hour at room temperature. They were then either transplanted into susceptible animals or explanted into tissue culture solid media. All tissues tested in vitro and in vivo at concentrations of 90 to 100 meq. per liter of either fluoride or oxalate ions survived without appreciable damage evidenced by good cellular proliferation and good function. If the concentration reached 140 meq. per liter of fluoride or oxalate ions, the tissues were irreversibly injured, manifested by the diminuty of cellular proliferation and complete loss of function. However, neoplastic tissues survived transplantation into susceptible animals as high as 600 meq. per liter of either fluoride or oxalate ions. The possibility

that fluoride and oxalate ions immobilize the same cations is being considered. The coincidence of equal milliequivalents per liter of fluoride and oxalate ions is emphasized.

45. Microorganisms From Saliva of Caries-Free and Caries-Susceptible Human Beings. *Gordon E. Green and Matthew C. Dodd, Dental Research Laboratory and Department of Bacteriology, Ohio State University, Columbus, Ohio.* Twenty-five caries-free and twenty-five caries-susceptible individuals were selected on the basis of clinical and roentgenologic examinations. Bacteriologic studies on the salivary flora of these two groups indicated the major difference to be in number and kind of lactobacilli. The average counts of lactobacilli are: caries-free, 2,000 per cubic centimeter; all smooth type, caries-susceptible, 400,000 per cubic centimeter of which 55 per cent were rough types. Further comparison of the cultural and biochemical properties of these lactobacilli disclosed that none of them grew appreciably in a medium of saliva, saline, and glucose. Smooth strains tend to become rough in liquid, but not in solid media. Saliva appears to reverse this tendency. In four media the rough strains have less acidogenic capabilities than smooth strains. From two caries-free subjects rough lactobacilli occasionally have been isolated. These organisms are different in cultural and acidogenic characteristics from rough strains of susceptible subjects. The caries-free rough strains have reverted to smooth only on laboratory media under certain conditions, and acidogenically they resemble the usual smooth strains. Growth curve experiments showed that filtered saliva from caries-free human beings only possessed an inhibitory capacity, inhibiting nine of ten strains tested. At present smooth strains of lactobacilli from caries-free and caries-susceptible human beings appear to be identical. In some physiologic characteristics rough strains from caries-free saliva closely resemble the smooth strains, while the caries-susceptible rough strains are entirely different from any of these. Certain of these variations can be influenced by the pH and composition of laboratory media. Experiments are in progress to relate these phenomena more closely to events taking place in saliva.

46. The Effect of Periodontal Treatment on the Band of Circular Fibers in the Marginal Gingiva. *David A. Hagerman and Sumter S. Arnim, Dental Branch, University of Texas, Houston, Texas.* Biopsies of the marginal gingiva and smears from gingival sulci were secured from a patient prior to, during, and following treatment of periodontal lesions by gingivectomy and by curettage, respectively. The sections were cut in horizontal plane so that the circular connective tissue fibers of the marginal gingiva were plainly revealed. It was found that these fibers were almost entirely destroyed in extensive periodontal lesions, and that they re-form following gingivectomy or curettage. Their presence and condition appeared to be related to the amount of bacterial plaque and other material found on the tooth near the gum margin. Clinical studies reveal that the tone of marginal gingiva and its adaptation to the tooth is largely dependent upon the presence and health of the band of circular fibers within the gingival margin.

47. Duplicability of Tests for Rate of Acid Production by Salivary Sediment. *G. L. Hargreaves and R. S. Manly, Tufts College Dental School, Boston, Mass.* When buffer, glucose, and hydrogen ion concentration and temperature are kept constant in a liquid that is in contact with sediment from whole stimulated saliva, the pH differential between the sediment and solution

at equilibrium is an index of the rate of acid production by the sediment. Inhibitors of acid production can be evaluated from their effect on this differential, using as the base the differential with a control test. The duplicability of this index appeared satisfactory with repetitions of inhibitors either on the same sediment mass, on different sediments from the same subject, or on sediments from different subjects. Results were similar with four inhibitors when comparison was made between the sediments obtained by brushing the teeth with water and the sediments obtained from the whole salivas of the same subjects. Several inhibitors were studied which have been proposed at one time or another for control of dental caries. The pH differential was decreased by 0.01 per cent sodium iodoacetate, 0.1 per cent sodium fluoride, and 0.01 per cent urea, but not by glyceraldehyde, naphthoquinone, or hydroquinone at these concentrations. No effect could be observed from treatment of a sediment with penicillin for several hours, even though the control differential from salivary sediment was diminished over one-half when saliva was collected twenty-four hours after brushing with penicillin dentifrice began.

48. The Cavity-Sealing Properties of Silicate Cement. *Irving T. Harris, Northwestern University Dental School, Chicago, Ill.* Cavities were prepared in teeth of a dog and filled as follows: (1) An arbitrary powder-liquid ratio which the operator regarded as being clinically acceptable. Cotton rolls were used to maintain a dry field and the cavity was desiccated with alcohol. A celluloid strip was held over the filling for five minutes; the cotton rolls were removed at the expiration of an additional ten minutes. After trimming off the excess, the filling was coated with cocoa butter. (2) Same as above with the exception that the slab temperature was controlled and the tooth isolated with a rubber dam. (3) Rubber dam; powder-liquid ratio 4 to 3; slab temperature 65° F. (4) Same, with the exception that the filling was coated with cocoa butter for twenty-five minutes before the margins were trimmed. The dog lived for thirty days on a normal dog diet. At the termination of this period, the teeth and fillings were kept moist wtih radioactive Ca^{45} for a period of two hours. Then, the animal was sacrificed and the teeth were sectioned. Radioautographs of the sections revealed the following: (A) Technic as outlined in (1): extensive penetration. (B) Technic as outlined in (2): penetration outlining the filling and extending well into the dentin. (C) Technic as in (3): filling well outlined by the tracer and considerable penetration of dentin. (D) Technic as in (4): some penetration of the Ca^{45}; however, this does not extend to the base of the cavity. Conclusions: The most significant changes from the standpoint of cavity seal were seen in the case in which cocoa butter was placed on the filling. It is suggested that only the most exacting procedures produce cavity seals of a fair degree of effectiveness.

49. The Cavity-Sealing Properties of Autopolymerizing Acrylic and Zinc Phosphate Cement. *Donovan W. Brown, Northwestern University Dental School, Chicago, Ill.* Cavities were prepared in teeth of a dog and filled as follows: (1) Autopolymerizing acrylic, pack method. (2) Autopolymerizing acrylic, brush method. (3) Zinc phosphate cement, "sloppy" mix. (4) Zinc phosphate cement, "thick" mix. The dog was allowed to live for thirty days on a normal dog diet. At the end of that period, the teeth containing the fillings were kept moist with radioactive Ca^{45} for a period of two hours. Then, the animal was sacrificed and the teeth were sectioned. Radioautographs of the teeth and fillings disclosed the following: (1) Pack acrylic: a large area of

penetration of Ca^{45} around the filling. (2) Brush acrylic: penetration of some magnitude, though less than around the pack acrylic. (3) Zinc phosphate cement ("sloppy" mix): a great amount of penetration. (4) Zinc phosphate cement ("thick" mix): markedly less penetration than in the case of the "sloppy" mix. Conclusions: (1) The brush method of placing acrylic produces a more effective cavity seal than the pack method. (2) If cement is to be used, either for cementation or as a cavity lining, it should be thoroughly mixed, and to as thick a consistency as is permissible for the operation.

50. Effect of Mixing Water in the Diet on the Development of Carious Lesions in Rats. *M. Rachel Harris and Robert M. Stephan, National Institute of Dental Research, Bethesda, Md.* Many investigators have reported differences in the development of rat caries with variations in the physical properties of the diet. In the experiments reported here we have mixed measured amounts of water into different diets in order to determine how the procedure would affect the production of caries in the white rat. These diets consisted of Basic Diets 580 and 593 and modifications made by the addition of different nutritional supplements or inhibitors. Each of these variations of the diets was run in parallel, (1) without added water, (2) with 5 ml. of water, and (3) with 15 ml. of water mixed with 100 Gm. of dry diet. Both diets and drinking water were given ad libitum. The addition of water in the diets resulted in (1) a decrease in the number of animals with caries—48.0 per cent for the dry diets, 22.2 per cent for the diets with 5 ml. of water, and 16.9 per cent for the diets with 15 ml. of water added; (2) a decrease in the average number of carious teeth per animal—2.87, 1.16, and 0.37, respectively; (3) a decrease in the average number of carious lesions on each of the tooth surfaces—occlusal, proximal, buccal, and lingual; (4) a decrease in periodontal destruction; (5) no change in incisor pigmentation; (6) an increase in calculus formation; and (7) an increase in average daily weight gain.

51. A Preliminary Report of the Effects of Urea and Chlorophyll on the Respiration of Saliva and a Method for Evaluating Various Samples of Chlorophyll. *Glen Hyden and John Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* By the Warburg technic, the respiration of saliva and the effects of various compounds on the respiration were studied. There are great differences in the rate of endogenous respiration of different samples of saliva. Further, the same sample of saliva changes its respiratory activity with the time and temperature at which it is kept. These variables make it difficult to compare results of work done at different times. After investigating the variations in respiration of different samples of saliva, the effects of urea and chlorophyll, in various concentrations and in combination at an effective concentration separately, on the endogenous respiration were studied. The effect of the compounds on the total endogenous respiration is expressed as the percentage of the control for a particular run, as a function of time. Then the effect of the compounds on endogenous respiration is expressed as the percentage of the rate of respiration of the control as a function of time. Both chlorophyll and urea, at appropriate concentrations, decrease the endogenous respiration. The combined effect of urea and chlorophyll shows that separate reactions in the respiration appear to be inhibited by the two compounds. Applying the same methods to various samples of chlorophyll, we find that, at the same concentrations, different samples of chlorophyll have different degrees of effectiveness in the inhibition of the endogenous respiration of saliva. This provides an additional method for determining the activity of various samples of chlorophyll.

52. Effect of Copper Sulfate on Initiation and Progression of Dental Caries in the Syrian Hamster. *John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* I have found that certain samples of copper chlorophyllins contain small amounts of loosely bound copper. The present experiment was done to learn whether or not this copper could account for the caries-preventive effect of copper chlorophyllin compounds. Thirty-seven 25- to 29-day-old Syrian hamsters from eight litters were placed by litter mate distribution into four groups and given drinking solutions containing copper as $CuSO_4$ and caries-producing diet ad libitum for 275 days. The resulting mean Keyes caries scores and standard deviations were: Group I, no copper, nine animals, 49 ± 44.3; Group II, 10 ppm Cu, nine animals, 25 ± 23.9; Group III, 25 ppm Cu, ten animals, 16 ± 9.8; Group IV, 50 ppm Cu, nine animals, 9 ± 11.7. Caries scores plotted against the logarithm of the copper concentration approximate a straight line, indicating tooth destruction was a function of the $CuSO_4$ concentration. Percentages of noncarious teeth in Groups I to IV were 11, 20, 28, 64, respectively, indicating $CuSO_4$ has an effect on initiation of decay, but not as a simple function of $CuSO_4$ concentration. This work gives support to the idea that initiation and progression of dental decay may be different mechanisms. It appears that minute amounts of unbound copper may explain the caries-preventive effect of copper chlorophyllins. Finally, since McClure (1948) found 250 ppm of Cu to have no pronounced effect on rat caries, it appears the mechanisms of dental decay in the rat and hamster differ sufficiently to cast doubt on a transfer of experimental caries findings between species.

53. The Caries Experience Rates of 12-, 13-, and 14-Year-Old School Children After Exposure to Artificially Fluoridated Water for 59 to 70 Months. *Iden N. Hill, J. Roy Blayney, and Walter Wolf, Zoller Memorial Dental Clinic, University of Chicago, Chicago, Ill.* The 12-, 13- and 14-year-old children examined in the Evanston Dental Caries Study in 1952, with 59 to 70 months' exposure to fluoridated water indicated a reduction in dental caries experience, expressed as per one hundred teeth, of approximately 19.50 per cent for the 12-year-olds, 20.22 per cent for the 13-year-olds, and 12.97 per cent for the 14-year-olds. These differences were noted when comparisons were made to children of the same ages examined in the base line group before the addition of sodium fluoride to the communal water. This variation in reduction may be attributed partially to the stage of eruption of the teeth at the time sodium fluoride was added to the water. At that time, February, 1947, this group of 12-, 13-, and 14-year-olds were then 7, 8, and 9 years of age. These children, with the crowns of their canines, premolars, and second molars fully calcified, had not reached the age at which these teeth erupt, but did so after the addition of sodium fluoride to the water. It is logical to assume that the greatest reduction in dental caries probably would be found in the 12-year group, as the canines and second molars are usually in the final stages of eruption at this time. However the 13-year-olds demonstrated a slightly greater reduction than the 12-year-olds. This may be due to the fact that at the time of examination the canine and second molars of some of the 12-year-olds had not yet erupted, while these particular teeth of the 13-year-olds were erupted but had not been exposed to the oral environment a sufficient length of time for caries to develop.

54. Preliminary Report on the Breeding of Caries-Immune and Caries-Susceptible Strains of Syrian Hamsters. *Harold C. Hodge, Erling Johansen, John W. Hein, Elliott A. Maynard, School of Medicine and Dentistry, Uni-*

versity of Rochester, Rochester, N. Y. To date three successive generations of hamsters have been studied for evidence of a hereditary pattern of caries susceptibility. The first generation was raised to 35 days of age on Purina Rabbit Pellets and then placed on a caries-producing diet consisting of 30 per cent whole wheat flour, 30 per cent whole powdered milk, 20 per cent starch, 15 per cent sucrose, 4 per cent alfalfa, and 1 per cent sodium chloride. After 100 days the carious involvement was scored according to the method of Johansen (*J. D. Res.* **31**: 361, 1952) and various combinations of caries-susceptible and caries-immune animals were mated. During the breeding and gestation period the starch and sugar were removed from the caries-producing diet and fresh vegetable supplements were added. The second generation animals were raised on this modified diet and placed on the caries-producing regimen at 35 days of age and maintained for 120 days. The carious lesions were then scored as previously indicated, and the mating and dietary procedures were repeated. The third generation was treated similarly to the second generation, maintained on the caries-producing diet for 119 days and then scored. From the data collected to date, it appears that there is a genetic influence governing dental caries in the hamster. This genetic influence has been deduced from the variation of the pattern of caries susceptibility between litters and the distribution of carious involvement within litters.

55. Staining Reactions Following Chelating and Other Decalcifying Methods. *H. A. Hunter, Faculty of Dentistry, University of Toronto, Toronto, Ontario.* The efficacy of a proprietary chelating agent (Versene) as a decalcifying medium for osseous and dental tissues was tested by using it and comparing the results with those of known materials. Seven series of experiments were set up using four to six guinea pig or rat mandibles and femurs in each set. They were arranged to test the effect of concentration, pH, and temperature of Versene solutions, and also the effect of ion-fixing resins, phloroglucin, and two fixatives—formalin and Zenker formol. These were compared with 5 per cent HNO_3, H.COOH—Na citrate, and Mg citrate solutions. Within the ranges of the experiment, concentration, pH, and temperature had no noticeable effect on the staining of Versene-decalcified tissues. However, the higher the concentration and temperature, the faster the rate of decalcification. There is a suggestion that the presence of ion-fixing resins may be detrimental to best results. The Versene and formic acid—sodium citrate-decalcified and formalin-fixed tissues consistently took more eosin and less hematoxylin (Harris) than did the Zenker formol-fixed tissues, but this difference did not apply in the cases of 5 per cent HNO_3 and 45 per cent formic acid-resin mixture. The 5 per cent HNO_3 with 1 per cent phloroglucin-decalcified tissues were by far the worst staining, appearing foggy and showing staining inconsistencies. It is suggested that the chelating agent (Versene) is a satisfactory high pH decalcifying medium and adaptable over a wide range of physical conditions and still permitting good routine histologic staining.

56. Clinical Experiences With Cortisone and ACTH in Five Cases of Periodontal Disease. *Rosa Iusem, L. Herráiz Ballestero, and Benito Monis, Instituto Municipal de Odontologia-Policlinicos Rivadavia y Penna, Buenos Aires, Argentina.* Cortisone has been found to be useful in: (a) diminishing capillary permeability, polymorphonuclear diapedesis, and inhibition of collagen; (b) reducing granulation plasma cells and lymphocytic proliferative tissues. The fact that these conditions are present in certain forms of periodontal disease, lead us to begin our investigations. Five patients were treated. Clinical color slides were taken in every case, and biopsy specimens from

control and treated areas in the fifth case. Case 1, a 33-year-old woman, had severe spontaneous gingival bleeding. Blood transfusions had to be administered after extractions. Hematologist's report showed no blood dyscrasia and normal clotting time. Cortisone was administered intramuscularly and was followed by dramatic clinical improvement. Five extractions were performed with no hemorrhage. Cases 2 and 3 were men aged 33 and 37, each suffering from periodontal disease with bone resorption, mobility, suppuration, and pockets. Gingival injections of cortisone were given. Case 4 developed a gingival tumor in the fourth month of pregnancy, with enlarged, bleeding desquamative gingivitis. Gingival injections with cortisone were given and the enlargement was reduced. Bleeding and desquamative conditions cleared up. Condition lasted for the remaining time of pregnancy. Case 5 was a woman aged 32 who had spontaneous severe bleeding of gums, gingival proliferation, desquamative gingivitis, and periodontal abscesses. No blood dyserasia or diabetes was present. The patient was put on a balanced diet and ACTH was administered intramuscularly after which she showed less bleeding. Cortisone was injected in the gingiva. The desquamative gingivitis disappeared and the proliferative tissues shrank. Conclusion: This clinical and pathologic study shows some beneficial action of cortisone to periodontal conditions where a systemic etiologic factor is present. It seems to work favorably upon enlarged, bleeding gingivitis and in reducing chronic inflammatory granulation tissue found in periodontal pockets and abscesses.

57. A Preliminary Report on the Effects of Ethylene Oxide on Saliva. *Sterling W. Jackson and Ned B. Williams, Department of Microbiology, School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* The antimicrobial action of gaseous or fluid ethylene oxide has been used effectively by several workers for the sterilization of culture media as well as of some of the antibiotics. The present work was initiated to determine whether ethylene oxide would effectively sterilize saliva, without having detrimental effects on certain other protein components of saliva, such as amylase. Ethylene oxide was added to stimulated saliva freshly collected from several human beings. The pooled saliva and all equipment employed was thoroughly chilled to about 5° C. The chemical was added to the saliva, and the tubes were placed at 5° C. for one hour, then overnight at 37° C. Sterility tests were made by inoculating duplicate sets of blood agar plates, chocolate agar plates, and tubes of brain-heart infusion broth. One set was incubated aerobically, the other anaerobically at 37° C for one week. Concentrations from 0.6 to 2.0 per cent volume sterilized all samples of saliva. It was noted that the pH of the saliva was increased in direct proportion to the amount of chemical added, but not sufficiently to affect the tests for sterility. Tests for amylolytic activity were made, in duplicate, before and after addition of ethylene oxide. Controls consisted of aliquots of the same pooled saliva to which no ethylene oxide was added. Amylolytic activity in the controls was practically eliminated, whereas about 80 per cent of the activity remained in saliva treated with 0.6 and 0.8 per cent by volume of ethylene oxide. Greater concentrations of the chemical reduced the amylolytic activity to a greater degree. The chemical seems to bind chloride ions since activity could be restored to some degree by appropriate replacement.

58. The Response of the Pulp of Dog, Monkey, and Man to Baseplate Gutta-Percha and Cavity Preparation. *Verda Elizabeth James, Isaac Schour, and John M. Spence, Departments of Histology and Operative Dentistry, Uni-*

versity of Illinois, College of Dentistry, Chicago, Ill. This histologic study is based on thirty-six human teeth, sixteen teeth of monkeys, and seventeen teeth of dogs, in which labiogingival cavities were prepared by bur or by airbrasive technic. The cavities were filled with baseplate gutta-percha and the teeth were extracted after periods ranging from three hours to thirty-six days. In control teeth and sections the normal pulp of the three species differed mainly in the morphology of the odontoblasts, being predominantly of the mature type in man, of the young type in the monkey, and of the young and preodontoblastic types in the dog. The pulpodentinal membrane appeared regularly in the normal human being and dog but was not regular in the monkey. Weil's cell-free zone was indistinct in the monkey and dog. The common experimental effects in the three types of teeth were (1) degeneration of odontoblasts and their replacement by young odontoblasts and preodontoblasts; (2) inflammation, first acute and then chronic, which persisted throughout the experimental periods; and (3) production of irregular dentin. The bur and airbrasive technics showed no significant differences in effects. The experimental species differences were: (1) After the second week the calciotraumatic line appeared regularly in monkeys and dogs, and in only one instance in man; (2) repair was more rapid in dogs than in monkeys and man; and (3) the irregular dentin formed more rapidly in the dog and least in man.

59. The Developmental Pattern of Dental Caries in the Syrian Hamster. *Erling Johansen, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* Three groups of 25- to 35-day-old hamsters, eighty-four males and eighty-one females, have been given periodic oral examinations for as long as 228 days on a caries-producing diet. The objective of the study was to determine (a) time of initiation of the carious process in individual molars, (b) the exact location of initial lesions, and (c) to follow the lesions progressively and determine the caries pattern of each tooth. The earliest gross carious lesions in the enamel were observed as follows: maxillary and mandibular second and third molars after 7 days; mandibular first molar after 38 days; maxillary first molar after 46 days. More commonly, carious lesions begin to develop as follows: in second and third molars between 20 and 50 days; first molars between 60 and 100 days. The most susceptible areas were as follows: maxillary third molar—the distolingual, distobuccal, and distoocclusal surfaces, and the mesiobuccal groove; maxillary second molar—mesiobuccal groove with involvement of occlusal fossa, and buccal groove; maxillary first molar—buccoocclusal grooves and smooth buccal and lingual surfaces; mandibular third molar—buccal groove with the lesion involving the distoocclusal fossa; second mandibular molar—the buccal groove; the mandibular first molar—the smooth buccal and lingual surfaces and the buccal and lingual-occlusal grooves. Small carious lesions in second molars predispose to fracture with frequent great loss of tooth structure. Plotting of successive caries scores (Keyes' system) against time shows the following general trends in caries susceptibility: (1) early initiation is usually associated with rapid progression, and high final score, but frequently exceptions occur; (2) later initiation also may show rapid progression, and high final score; and (3) late initiation is usually associated with slow progression and low final score.

60. Histochemical Studies of the Calcifying Tooth. II. *Percy L. Johnson and G. Bevelander, New York University College of Dentistry, Department of Histology, New York, N. Y.* Certain correlations may be derived from the histochemical observations to be made here with those previously reported and described by us. (1) The localization of nucleic acids in the odontoblast

and ameloblast parallels that described for the localization of alkaline phosphatases. The fact that ameloblasts are concerned with the elaboration of the organic matrix of enamel correlates well with other previous observations that nucleic acids may be associated with the elaboration of protein. For odontoblasts this correlation is not as clearly evident. The interrelation between the presence of nucleic acid and alkaline phosphatase in the ameloblasts and odontoblasts also lends support to the supposition that the presence of phosphatases in these cells may well be related to the metabolism of the nucleic acids. (2) In regard to calcifying tissues of the tooth an important correlation appears when an examination of the initiation and degree of calcification of dentin is compared with the presence and intensity of mucopolysaccharides. In this tissue calcification appears to parallel the increase in the amount of mucopolysaccharides present in the dentinal matrix. (3) The correlation between the observed metachromasia and calcification in dentin is somewhat more difficult to interpret. The ground substance in which Korff's fibers are imbedded, as well as in predentin, exhibits strong metachromasia, indicative of the presence of acid polysaccharides which we believe to be chondroitin sulfate. The loss of metachromasia in calcification coincident with the appearance of calcospherites which are orthochromatic at first appears to be somewhat puzzling. One possibility which might explain the loss of metachromasia (chondroitin sulfate) and the appearance of orthochromasia could result from a change in the concentration of chondroitin sulfate. This would further explain the complete loss in any tinctorial reaction of the more highly calcifying dentin. On the basis of these metachromatic studies it is tentatively suggested that acid polysaccharides gradually increase as calcification takes place. As the tissue becomes more highly calcified chondroitin sulfate appears to be diminished or lost. It would appear, therefore, that during the process of calcification acid polysaccharides are part of the ground substance of dentin, and as calcification is completed these components are either lost or converted into other substances and thus are no longer detectable by our analytic methods.

61. Histopathology of Irradiated Salivary Glands. *Wm. J. Kasboum, Dental Branch, University of Texas, Houston, Texas.* Salivary glands secured from radical neck dissections following irradiation were studied microscopically. The primary neoplastic lesions were on the lip or in the mouth. Surgical dissection of the glands was performed from one week to two years following irradiation. Sialadenitis was seen in the early cases. Atrophy with fatty metamorphosis of the glands followed irradiation. These changes were influenced by the time interval between irradiation and surgery and the age of the patient. A decrease in salivation was noted in all patients. Teeth were removed before irradiation. Regeneration of the salivary glandular epithelium was not seen in any instance.

62. An Injection and Clearing Procedure for Visualization of the Blood Vessels Within Soft and Calcified Tissues. *Gerard J. Keller, Sylvio Bevilaqua, and D. Walter Cohen, Department of Oral Histopathology, School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* A number of substances (latex, gelatine masses, and India ink) have been employed for the injection of blood channels, but most fail to produce consistently satisfactory results. The reasons are apparent when smears of these masses are microscopically examined. To demonstrate capillaries with any degree of certainty, none of the particles of the injection mass can be greater than 7 micra in diameter (the approximate size of the red blood cell). Particles 50 to 60 micra in size are not

uncommonly found in the usual injection material. Our efforts were directed toward refinement of the injection material. India ink was subjected to vacuum filtration, centrifuging, repeated vacuum filtration, and microscopic examinations during various stages of the procedure. The final product has particles less than 1 micron in diameter. Perfusion with normal saline solution previous to injection is essential if satisfactory results are to be obtained. Injections, as well as perfusions, were carried out at 75 mm. mercury. Injected specimens were fixed in 10 per cent formalin for about two days. They then were sectioned on a hard tissue cutting apparatus. This instrument permits serial cutting of undecalcified material. The sections (100 micra to 1 mm. in thickness) then are dehydrated in alcohol and cleared in a mixture of methyl salicylate 50 c.c., benzyl benzoate 100 c.c., and carbolic acid, 150 c.c. This method is being used by us for extensive studies of the vascular patterns of oral tissues. Perfusions and injections are being done via the common carotid artery of the moribund animal.

63. Moderate Caries Activity in Syrian Hamsters. *Paul H. Keyes, Harvard School of Dental Medicine, Harvard University, Boston, Mass.* A moderate degree of dental caries has been found in a series of seventy-three hamsters fed, during the period of tooth development, purified diets containing whole wheat ash as all, or part, of the source of minerals. The diets contained sucrose, 60-65 per cent; casein, 21 per cent; corn oil, 5 per cent; liver concentrate, 5 per cent; whole wheat ash, 2 to 4 per cent; vitamin supplements; and Celluflor, 8 Gm. per 100 Gm. The most frequently employed postdevelopmental diet contained whole wheat flour, 30 per cent; whole powdered milk, 30 per cent; confectioner's sugar, 15 per cent; corn starch, 20 per cent; alfalfa, 4 per cent; NaCl, 1 per cent. The numbers of lesions have varied from none to approximately twenty, and scores from none to approximately forty-five. These results are in marked contrast to previously reported data (*J. D. Res.* **31**: 477 Abstract 49, 1952) in which there was almost complete absence of caries in animals maintained under the same extremely well-controlled environmental conditions, but fed purified diets supplying the mineral fraction in the form of either pure mineral salts or the ash of Purina Laboratory chow.

64. Some Neglected Factors in the Interpretation of Metachromatic Staining of Tissues. *Grace C. Kimball, Western Reserve University, Cleveland, Ohio.* The interpretation of the appearance of metachromasia in stained sections of injured connective tissue is, at present, quite confused. In vitro studies with some of the mucopolysaccharide of the ground substance show that concentration, redox potential, and light may determine the presence or absence of metachromasia. These are not new facts, but their application to the study of connective tissue diseases has been overlooked.

65. Dental Caries in Desalivated Hamsters Maintained on a Starch Diet. *C. E. Klapper, University of Alabama, Schools of Medicine and Dentistry, Birmingham, Ala.* Preliminary studies on the influence of salivary glands on the development of experimental dental caries have been reported from these laboratories. It has been established that little or no dental caries will result in animals placed on a cariogenic diet after they have reached an age of 35 days. In contrast, partially desalivated adult animals show definite tooth lesions when placed on a high-sugar cariogenic diet. Only minimal caries are found when partially desalivated adult animals are placed on a cariogenic diet in which starch replaces the sugar. Partially desalivated animals are those in which

either the submaxillary and sublingual glands have been removed or the parotid duct has been ligated and severed. The investigation has been expanded by completely desalivating a group of twenty-four Syrian hamsters. At approximately 40 days of age they were placed on a modified Hopper, Webber, Caniff diet made up of 60 per cent cornstarch, 36 per cent whole milk, 3 per cent alfalfa meal, and 1 per cent sodium chloride. All animals were sacrificed 115 days later. Fairly extensive caries were found in the teeth of all of these animals. An average of nine carious teeth were present. The average total number of caries was fourteen per animal. These results establish the fact that definite and extensive carious lesions develop in the molars of hamsters in which the only source of saliva is from the small intramural glands of the oral cavity and maintained on a diet with a high concentration of starch. It has been established previously that the type of saliva is not important in the development of experimental dental caries. In animals maintained on the same diet in which only the parotid glands were left intact, the caries score and pattern was similar to that of animals in which the submaxillary and sublingual glands were the only normally functioning major salivary glands. The findings of the present experiment lend added support to the hypothesis that the mechanical cleansing action of the saliva is of paramount importance in the elimination of production of experimental dental caries by a starch diet.

66. Effect of Paraffin-Stimulated Saliva on the Growth of Escherichia Coli. *Holmes T. Knighton, Department of Bacteriology and School of Dentistry, Medical College of Virginia, Richmond, Va.* Approximately 4,000 *Escherichia coli* cells, contained in 0.1 ml. amounts of diluted broth cultures, were added to 4 ml. lots of saliva. After 20 hours' incubation (37° C.), 0.1 ml. lots of varying dilutions were evenly distributed over E.M.B. agar plates. *E. coli* colonies were counted after 24 hours' incubation. Samples of saliva, all originally negative for *E. coli,* were obtained from fifty individuals. Tests were made with (A) whole saliva—(1) untreated, (2) containing 1 per cent glucose, (3) containing 1 per cent peptone; (B) Berkefeld filtered saliva. Increase of *E. coli* was noted in all samples except (1) untrealed whole saliva (no growth in 40 per cent of samples), and (2) whole saliva containing glucose (no growth in 98 per cent). Acid produced by the oral flora probably accounted for the latter result. The average increase in *E. coli* was approximately: (A) whole saliva—(1) untreated, 100 fold, (2) containing 1 per cent glucose, none, (3) containing 1 per cent peptone, 16,000 fold; (B) filtered saliva—(1) untreated, 100,000 fold, (2) containing 1 per cent glucose, 790,000 fold, (3) containing 1 per cent peptone, 950,000 fold. No evidence of specific antibiotic effects was noted. Results suggest a competition for media as one of probably numerous factors limiting colonization of transient contaminants in the mouth.

67. Revision of Dental Regime to Prevent Subacute Bacterial Endocarditis. *Frederick W. Kraus, Tufts College Dental School, Boston, Mass.* According to prevailing professional opinion, not more than one to three teeth should be extracted at one time in patients with valvular damage. This opinion follows the advice given in eleven scientific publications. In ten among these the advice is speculative, in one it is based on obsolete experimental technic. We propose a reversal of this policy for the following reasons: (1) Bacteremia, the accepted link between tooth removal and subacute bacterial endocarditis accompanies single and multiple extractions with equal frequency, duration, and probably magnitude. (2) Among fifty-eight cases of s.b.e. referable to preceding tooth extractions, one tooth was extracted in each of twenty-six patients, two

teeth in six and several teeth in twenty-six patients. These numbers show no correlation between causation of s.b.e. and the number of teeth removed. (3) The patients must receive protection by sulfonamide or antibiotic premedication. No one should be exposed to the action of these drugs more often than necessary. Conclusion: since every tooth extraction seems to be accompanied by bacteremia, although not every bacteremia can be detected, it is logical to reduce the risk of bacteremia by reducing the number of operations, rather than the number of teeth extracted per sitting.

68. Changes in the Teeth of Rabbits Infected With Vaccinia Virus. *Seymour J. Kreshover, O. Wendell Clough, and James A. Hancock, Medical College of Virginia, Richmond, Va.* In the course of experimental studies to determine the effect of various maternal virus diseases on fetal dental development, striking gross and microscopic abnormalities were noted in a group of sixteen parent rabbits that had been inoculated intravenously with 1 ml. doses of vaccinia virus suspensions titering 10^{-4} to 10^{-6}. Manifestations of generalized infection were evidenced in nine animals by the appearance of lip and nasal lesions three to four days following virus injection. Although the remaining seven rabbits failed to demonstrate similar lesions, neutralizing antibodies were present in their blood at time of sacrifice. Periods of animal observation ranged from 35 to 330 days postinoculation. In six cases, marked degrees of macroscopic enamel hypoplasia became evident at about 160 days. Histologic study showed similar changes in the unerupted portions of the incisor teeth of all but four of the remaining animals. Abnormalities of the pulp and dentin also were noted. The observed changes were characterized chiefly by marked irregularity of the enamel surface due to numerous invaginations by degenerated and atrophic ameloblastic cells; corresponding wavelike irregularities at the dentinoenamel junction and pulpal surface of predentin; and retrogressive changes in the pulp. A series of noninfected control animals failed to demonstrate any abnormalities.

69. A Correlated Study of Facial Growth During the Period of the Changing Dentition. *Wilton Marion Krogman, Graduate School of Medicine and Evans Institute of Dentistry, University of Pennsylvania, Philadelphia, Pa.* A preliminary report on the study of 600 Philadelphia school children (300 boys and 300 girls) seen annually since 1948, serving as "normal" controls. Also 500 orthodontic cases, most of whom were seen semiannually since 1947-48 and 1948-49. On each of the School Series the head and face were measured in careful detail: (1) cephalometry, or ecto-oral; (2) palatometry, or endo-oral (measured directly on the palate); and (3) roentgenographic cephalometry, measured on x-ray tracings (last three years only). All data are handled statistically on the basis of three ages: (1) chronological; (2) dental, or eruptive; and (3) skeletal. When the data on the School Series were pooled it was found that ecto-oral depths are more related to palate length than ecto-oral breadths are to palate breadth. Mesiodistal relationships in the face are more prognostic of dento-palato-facial relationships than transverse relationships. It is concluded that this interpretation validates the use of the lateral headplate in the analysis of faciodental relationships. When the data were grouped according to orthodontic classification (Angle) an important dimensional fact emerged: In palate length little or no difference was found in Normal, Class I, and Class II, Division 1, malocclusions. This suggests that malocclusion is not so much in differential palate growth, per se, but rather in the relationship of palatal arch to cephalofacial structure.

70. Comparative Anatomy of the Mucosa of the Tongue and the Palate of the Laboratory Mouse. *Helen Kutuzov, Department of Histology, University of Illinois College of Dentistry, and Harry Sicher, Department of Anatomy and Histology, Loyola University, School of Dentistry, Chicago, Ill.* The palates and tongues of fifty mice of four different laboratory strains were examined macroscopically and microscopically and the observations were compared with similar studies on the white rat. The tongue of the mouse was found to differ from that of the rat only in minor details: (1) the giant papillae are two-pronged in the mouse and simple in the rat; (2) the filiform papillae end commonly in two secondary papillae in the mouse, three to five in the rat. The palate of the mouse was found to differ widely from that of the rat, especially in the intermolar area: (1) it is relatively wider in the mouse than in the rat; (2) the pattern of the intermolar rugae is quite variable in the mouse compared to the rigid pattern of the rat; (3) the intermolar rugae of the rat are composed of strongly hornified secondary ridges, while those in the mouse are uniformly smooth; (4) the ratio between the occlusal molar area and the intermolar palatine area differs strikingly in the mouse (1:1) and in the rat (1:2); (5) the postrugal field of the mouse lacks the numerous fine papillae characteristic for that area in the rat. Despite the lower level of differentiation of the intermolar rugae and lack of screening area in the mouse, its total masticatory efficiency seems to be at least the same as that in the rat, mainly due to the increase in the relative size of the occlusal surfaces of the molars.

71. An Objective Method for Determining Deodorant Capacity. *L. L. Langley and H. S. Polin, University of Alabama School of Dentistry, Birmingham, Ala.* The dental practitioner is being subjected to many claims regarding the efficiency of oral deodorants. Currently the most popular method of determining the deodorant capacity of any substance involves the mixture of that material with an odoriferous substance. The resulting vapors then are evaluated by a subjective method using an osmoscope. In view of the fact that the olfactory receptors rapidly adapt to most odors, it is obvious that the osmoscope is plagued with definite limitations. A method developed in these laboratories consists of a recirculating system into which the odoriferous gas can be introduced, and samples for analysis easily withdrawn. By the use of a series of valves it is possible to recirculate the gas through the system without its coming in contact with the deodorant material. After control values are obtained, the valves are turned, causing the gas to pass over the deodorant substance. Following a standard period of time, samples are withdrawn, analyzed, and compared with the control values. The deodorant used in the initial tests of the method was potassium sodium chlorophyllin. The odoriferous gas consisted of a mixture of hydrogen sulfide and oxygen. The gas samples for analysis were withdrawn by suction through water in which the hydrogen sulfide dissolves. The water solution then was titrated with a standard iodine solution using a starch indicator. The per cent reduction in the hydrogen sulfide concentration depends upon the time of circulation, the temperature of the gas, and the concentration of the chlorophyll. Under standard conditions results agree within 5 per cent.

72. Cultivation of Rat Molar Tooth Germs. *William Lefkowitz, Charles F. Bodecker, and Dorothy F. Mardfin, Dental Department, Montefiore Hospital, New York, N. Y.* The in vitro culture of tissues as an experimental method has provided a valuable technic in experimental biology. The

potentialities of the method should be explored further by dental investigators. The rat was chosen as the experimental animal. Preliminary investigations established the in situ histologic development of odontogenesis in a 24-hour series. Two hundred eighty-three tooth germs were dissected from 17- to 21-day rat embryos. The technic of dissection is described. Forty sections of mandibles and maxillae containing tooth germs and seventy-nine dissected tooth germs were cultivated. The media investigated contained 75 per cent chicken plasma and 25 per cent of varying concentrations of both chicken and rat embryo extract. Cultivation of sections of the mandible and maxilla were unsuccessful. The dissected tooth germs showed growth and development. The tooth germs cultivated in the combination of chicken plasma and rat embryo extract were better than those cultured in chicken plasma and chick embryo extract.

73. Tracer Studies on the Uptake of Cadmium in the Rat Molar. *Henry M. Leicester, Paul R. Thomassen, and George J. Denzler, College of Physicians and Surgeons, San Francisco, Calif.* Two groups of rats were used in this experiment. One group was placed upon Cd^{115} in drinking water from time of conception until time of sacrifice. The other group received intraperitoneal injection of Cd^{115} from date of birth until time of sacrifice. Animals from each group were sacrificed at 25 and 68 days, and the molars were removed, dried, and ground to a fine powder for assay under the Geiger-Mueller tube counter. Other molars were sectioned to tissue paper thickness and placed against x-ray film in a suitable press for radioautographic studies. Molars from rats on drinking water had a significantly higher uptake of Cd^{115} than molars from rats receiving the Cd^{115} parenterally. Radioautographs showed this uptake to be mainly upon the surface of the tooth. This would lead one to believe that cadmium uptake in the rat molar is primarily that of adsorption rather than of chemical combination in the apatite lattice of the tooth.

74. The Staining of Dental Tissues Using Polyhydroxycarboxylic Acids and Iron. *Barnet M. Levy, Columbia University, School of Dental and Oral Surgery, New York, N. Y.* Sections of tissues from the mouth and jaws of various animals were treated with either tannic acid (M-digallic), pyrogallic acid, gallic acid, resorcylic acid, catechol, orcinol, pyrogallol, resorcinol or phloroglucinol for various periods of time, and subsequently treated with ferric chloride or ferric ammonium sulfate. Some of these compounds gave good intercellular staining, others none at all. While the cytochemical implications have not yet been completely worked out, such excellent preparations of periodontal fibers, bone, and teeth, as well as lamina propria fibers and striated muscle, may be made by using a simple technic that the method is presented at this time. The technic follows: (1) Prepare Bouin's fixed material as usual and run sections down to water. (Bouin's fixation is best, but any fixative may be used.) (2) Treat sections five minutes in a solution of tannic acid, 10 Gm.; acetic acid, 20 c.c.; distilled water, 100 c.c. (3) Wash well. (4) Place in 10 per cent aqueous solution of ferric ammonium sulfate for three to thirty seconds. (5) Wash, dehydrate, and mount as usual. Sections may be counterstained with picric acid, eosin, fast green, etc. Credit should be given to Salazar for applying the old tannic acid-iron technic to mammalian tissue. A complete bibliography can be found in his paper in *Stain Technology* **19**: 131, 1944.

75. The Effect of Fluorine on the Metabolism of Calcium and Phosphorus in the Rachitic Rat. *R. C. Likins, I. Zipkin, F. J. McClure, and A. Steere, National Institute of Dental Research, Bethesda, Md.* Severe rickets was induced in female Sprague-Dawley rats by feeding the phosphorus-deficient diet of Coleman, Becks, et al. During an induction period of twenty-one days one-half of twenty-four litter pairs received a total of 7.5 mg. of fluorine (NaF) administered by mouth in fifteen equally divided doses; corresponding pair mates served as controls. On the twenty-first day and each of four successive days all animals were administered radioactive calcium and phosphorus intraperitoneally. Twenty-four hours after the final injection, the animals were sacrificed and the femurs and mandibles dissected free. The tissues were dried to constant weight, ashed, and subjected to radiochemical analysis according to standard procedures. Per milligram of ash, the mean percentages of administered dose retained by control and fluorosed femurs, respectively, are: For Ca^{45}, 0.090 and 0.092; for P^{32}, 0.106 and 0.108. Similarly, the mean percentages retained by the mandibles are: For Ca^{45}, 0.069 and 0.074; for P^{32}, 0.073 and 0.074. The data indicate that the ingestion of approximately 17.2 mg. of F per kilogram of body weight did not appreciably alter the net uptake of Ca^{45} and P^{32} in the femurs and mandibles of these rats.

76. Streptococcal Beta Glucuronidase and Hyaluronidase. *Phyllis L. Lorina and Vincent F. Lisanti, Tufts College Dental School, Boston, Mass.* Investigations into the production of enzymes by oral bacteria were instituted because of correlations found between dental conditions and the beta glucuronidase and hyaluronidase titers of saliva. Beta glucuronidase activity has been reported in whole and parotid saliva. Preliminary experiments have shown that oral bacteria were capable of elaborating these enzymes. Although numerous microorganisms have been reported as producing beta glucuronidase, none have been isolated from the oral cavity. In isolating the salivary bacteria, which were capable of producing beta glucuronidase, a comparative survey also was run on other streptococci not of oral origin. To date, the bacteria found to produce this enzyme were all *Streptococcus mitis*. Among the microorganisms tested, which were not of salivary origin, only *Streptococcus uberis* and a streptococcus of Lancefield group L were found to elaborate this enzyme. Hyaluronidase-producing microorganisms isolated from saliva were found to be predominantly *Streptococcus mitis*, with streptococci of Lancefield groups A and K also present. These organisms were also tested for beta glucuronidase activity but did not demonstrate the presence of this enzyme. Of twenty-four strains of *Streptococcus mitis*, which were hyaluronidase producers, fifteen were found to have beta glucuronidase activity. *Streptococcus uberis* and Lancefield group L organisms isolated primarily as beta glucuronidase producers also were found to elaborate hyaluronidase.

77. Laboratory Methods for Evaluating Local Anesthetic Solutions. *F. P. Luduena, J. O. Hoppe, M. L. Tainter and G. D. Wessinger, Sterling-Winthrop Research Institute, Rensselaer, N. Y.* The three most important properties of a solution designed for local anesthesia in dentistry are its potency, toxicity, and irritation at the site of injection. Many different methods for determining potency have been described in the literature, but many of these do not give a reliable index of clinical performance. Since an injected anesthetic solution will produce anesthesia as long as its concentra-

tion remains above the threshold value, a test to determine the maximum amount of dilution that the solution can stand before losing its anesthetic effect should give a reliable index as to its potency. A modification of the Bulbring and Wajda method has shown considerable promise in our hands. The precise method for determining the threshold anesthetic dilution of clinical solutions is described in detail. Toxicity determinations by various routes of administration are the conventional ones carried out under carefully standardized conditions. Since solutions used in the clinic may contain several active constituents in addition to the local anesthetic agent, the LD_{50} should be expressed in terms of volume of solution, rather than by weight of the pure anesthetic compound. Local irritation is determined by modifications of the trypan blue method under carefully standardized conditions. The values obtained for these various properties according to the methods described should give a reliable and accurate index to clinical performance.

78. Further Observations Upon the Caries-Producing Potentialities of Various Foodstuffs. *Thomas G. Ludwig, Margaret R. Dewar, and Basil G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* Variations that occur in the amount of various foods retained in the mouth as estimated by the carbohydrate present in oral rinsings were made by the Somogoyi-Schaffer-Hartmann method and the quicker and more accurate anthrone method. The variations that occurred in the retention values for different individuals and for the same individuals from time to time were determined in the tests on a variety of foods, including several types of carbonated beverages. The effect of carbonation and acidulation upon the retention of the beverages was also studied. Considerable variations were found in the values given by different individuals and could not be directly related to any particular feature of the individuals' eating habits or oral conditions. The variations in the retention of a given food by one individual from time to time were sometimes large enough to overshadow the differences that occurred between various foods.

79. The Influence of Injury to the Periodontal Membrane on the Spread of Gingival Inflammation. *Luz C. Macapanpan and Joseph P. Weinmann, Departments of Oral Pathology and Histology, University of Illinois College of Dentistry, Chicago, Ill.* The progress of gingivitis of local origin into the periodontal membrane damaged during experimental tooth movement was studied in serial histologic sections of the molar teeth and supporting structures of thirty-five male young adult rats. Tooth movement was induced by the insertion of a piece of rubber dam between the upper right first and second molars which was allowed to remain in place from one to seventy-two hours. The rubber dam acted also as a foreign body and caused papillary gingivitis. In the first twelve hours, the insertion of the rubber dam caused compression of the gingival papilla. A small number of neutrophil leukocytes infiltrated immediately beneath the gingival epithelium or deeper into the connective tissue close to the alveolar crest. Between twenty-four and seventy-two hours, the gingivitis had increased in severity and the leukocytic infiltration had spread (1) directly into the periodontal membranes injured by tension on both sides of the interdental septum between the first and second molars, and (2) mesially into the lingual marginal and attached gingiva into the periodontal membrane of the intermediolingual root of the first molar. Measurements of the periodontal membrane showed that the magnitude of movement of the first molar was greater than that of the

second molar, leading to more extensive damage of the periodontal membrane of the first molar than those of the second and third molars. Leukocytic infiltration spread deeper into the more severely injured periodontal membrane of the first molar. The periodontal membranes distant from the gingival injury showed no signs of leukocytic infiltration. If the periodontal membrane is normal, the inflammation will spread into the bone marrow. If the suspensory apparatus is injured by tension, the inflammation might spread directly into the periodontal membrane. Injury from tension alone will not lead to leukocytic infiltration.

80. Further Studies of the Relationship of Personality Variables to Dental Caries. *John H. Manhold, U. S. Naval School of Aviation Medicine, Pensacola, Fla.* In 1949 Manhold and Manhold presented a preliminary report on the relationship between personality variables and dental caries. This present study expands the former work by using 266 subjects and varied tests. Guilford-Martin and Bernreuter Personality Inventories were administered to eight classes of Naval Aviation Cadets. Scores were obtained for all Guilford-Martin traits and for Bernreuter traits studied earlier. The mouths of the cadets were charted, both visually and roentgenographically, and DMF scores were obtained. Correlations between personality scores and DMF were then calculated. Bernreuter-DMF correlations of –.03 for neurotic tendency and –.02 for introversion-extraversion for the total population were not significant. Correlations of DMF with the Guilford-Martin subtests of agreeableness, cooperativeness, and objectivity, designated as the "psychotic triad," were significant. These coefficients for the total population were –.13, –.12, and –.07, respectively. The agreeableness and cooperativeness scores are significant at the 5 per cent level of confidence. The objectivity correlation is not significant at this level, but is in the same direction giving a positive trend to the triad. A fourth trait, ascendency or leadership, presented a correlation coefficient of +.12, a score also significant at the 5 per cent level. Inspection of individual class correlation coefficients of these traits also appeared to substantiate total population results. It appears, therefore, that some type of relationship exists between DMF and certain personality variables, although the underlying psychological meaning of these correlations is not clear.

81. A Method for Study of Acid Production by Salivary Sediments. *R. S. Manly, Tufts College Dental School, Boston, Mass.* A coherent mass of oral microorganisms and debris was obtained by centrifugation of whole, stimulated saliva at 3,000 r.p.m. for 20 minutes. One milliliter of the gelatinous precipitate was coated on a glass electrode and immersed in a solution at 37° C., containing 0.01 molar bicarbonate buffer and 0.2 molar glucose. The pH of the sediment usually dropped 2 units below that of the buffer solution within 20 minutes. A second glass electrode recorded the pH of the buffer solution. Both electrodes were connected to a Model R Beckman pH meter which operated through an automatic switch to give automatic recording of pH on a Brown six-place recorder. Under these conditions it was found that equilibrium is attained within a few minutes. The pH of the sediment reaches a constant value which changes only as the sediment loses activity. Apparently the equilibrium represents a balance between rate of acid formation and rates of diffusion of acid outward and buffer inward. Equilibrium can be disturbed or changed by agitation of the external solution, change of temperature, pH or buffer capacity of the solution in contact with the sediment, or introduction of a substance capable of inhibiting acid formation.

82. A Method for the Cultivation of Tooth Germs in Carrel Flasks. *Dorothy F. Mardfin, William Lefkowitz, and Charles F. Bodecker, Dental Department, Montefiore Hospital, New York, N. Y.* The in vitro culture of tooth germs is a problem of organized growth. Previous work in this field has been done by the hanging drop method, watch crystal technic, and in a depression slide. These methods present limitations for the cultivation of a whole organ. The Carrel flask technic has the following advantages for in vitro study of the tooth germ. It permits the use of from 1.0 ml. to 2.0 ml. of nutrient medium. The tissues may be washed and additional fluid added without transfer to a new clot. The pH may be observed and adjusted with ease. Better microscopic observation is possible. The technic of planting, washing, pH adjustment, and histologic processing is described.

83. A New Instrument for Recording Dental and Oral Muscle Forces: The Photoelectric Myodynagraph. *Herbert I. Margolis and Prem Prakash, Tufts College Dental School, Boston, Mass.* Imbalance of dental environmental muscle forces as an etiologic factor in malocclusions has been widely discussed in clinical orthodontics. However, scientific approach to this problem has been limited due to the nonavailability of a suitable instrument for recording these muscle forces with precision and accuracy.

The photoelectric myodynagraph, specially designed for this purpose, has an enclosed column of air at atmospheric pressure connected to a photoelectric recording mechanism. It consists essentially of five components: (1) Molded rubber mouthpieces (for lips, 8 mm. in diam., 3.5 mm. in thickness; for tongue, 8 mm. in diam., 4.5 mm. in thickness), connected by incompressible polyethylene leads to (2) two three-way stopcocks which are connected to the (3) basic instrument of the photoelectric recorder. Pressure applied on the mouthpiece is transmitted through the column of air to the rubber diaphragm and causes the mirror of the basic instrument to rotate around its axis. The beam of light from the photoelectric system is deflected by this rotation of the mirror which in turn activates (4) the photoelectric mechanism of the recorder and the magnitude of pressure is recorded on a moving roll of paper. (5) A specially designed calibrator permits recordings to be converted in terms of force per unit area. This instrument is being used at present to study (with adequate control groups) lip and tongue forces of groups of children with different dentofacial anomalies, receiving orthodontic treatment and/or myofunctional therapy. The range of this instrument is from 2 to 2,000 Gm., with an average 6 per cent range of error. It is not influenced by fluctuations of line voltage or by atmospheric changes within and outside the oral cavity. Its range or sensitivity can be changed by substituting different mouthpieces or by adjustment of the basic instrument of the photoelectric system.

84. Ameloblastic Changes Compared With Hemoglobin Concentrations in Rats Drinking Minimal Amounts of Fluorine. *Morris M. Matt, Department of Oral Pathology, Western Reserve University, Cleveland, Ohio.* Clinical studies have led to the assumption that the ameloblasts are most susceptible to the toxic action of fluorine. It has been noted that in experimental chronic fluorine intoxication of young dogs, histologic changes of the bones occurred prior to change in the tooth buds. Other investigators have reported the toxic effect of chronic fluorine ingestion upon the hemoglobin content of the blood in the rat and the iron content of the rat incisor. Forty-eight freshly weaned rats were divided into four equal groups. Varying amounts of fluorine were added to the water supply of the animals. Hemoglobin determinations

were performed at the start of the experiment, on the thirtieth experimental day and the sixtieth experimental day. On the thirtieth experimental day, sample rats from each group were killed and prepared for histologic examination. At thirty days, the average hemoglobin value for each group was inversely related to the fluorine content of the water supply. A depression in hemoglobin content occurred prior to histologic changes in the ameloblasts. This hemoglobin depression is transitory, and hemoglobin content returned to original values on the sixtieth experimental day. These results cast doubt upon the assumption that the extrasensitivity of the ameloblasts of the incisors in the growing rat is the first response to the toxic action of fluorine.

85. Anatomic and Functional Studies of the Temporomandibular Joint in Relation to Jaw Repositioning. *John Oppie McCall, New Organization School for Graduate Dentists, New York, N. Y., and Chester A. Swinyard, Department of Anatomy, University of Utah, College of Medicine, Salt Lake City, Utah.* It has been commonly held that the relation of the mandible to the maxilla is not subject to corrective change. This belief is based on the hypothesis that jaw position is dependent on condyle-fossa relations and that the usual (retruded) position of the condyle in the fossa may not be changed. A further related postulate is that the mandible is a lever of the third class and that the temporomandibular joint is a stress-bearing joint. Anatomic studies indicate that this joint is not stress bearing. Also, the wide range of movement of the articulating members of the joint supports the contention that it is not stress bearing. It further has been found that mandibular position and functional range are not entirely controlled by condyle-fossa relations. Anatomic and neurological studies indicate that mandibular position is primarily under muscular control, this being regulated by neuromuscular reflexes. It therefore follows that mandibular position can be changed, if unfavorable, through muscle re-education together with provision of new properly related articulating surfaces, even though this involves changing the position of the condyle. Clinical experience indicates that correction of jaw malposture provides relief of pain and other symptoms which often are related to stresses imposed by abnormal mandibular position. In cases so treated symptoms have been relieved and the new position and function of the mandible have become habitual.

86. The Inhibitive Effect of Dietary Phosphate Fertilizer on Dental Cariogenesis. *J. F. McClendon and J. Gershon-Cohen, Albert Einstein Medical Center, Northern Division, Philadelphia, Pa.* Studies were made in rats of the effects on cariogenesis by the addition of phosphate fertilizer directly to the diet. X-ray examinations of the teeth, jaws, and skeleton were included as part of these studies. The findings revealed retardation of skeletal decalcification, a life-sparing effect on the animals, and marked cariostasis.

87. The Effect of X-ray Irradiation Upon the Oral Tissues of the Macacus Rhesus. *Herman Medak, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* Studies have been made of the effect of x-ray irradiation on the oral tissues of the *Macacus rhesus*. A General Electric Two-Fifty Maxitron operated at 250 kv., 30 Ma., 15 cm. standard, with 1 mm. aluminum and 0.5 mm. copper filters, HVL 1.3 mm. Cu and 3 cm. round portal, was used to administer 600 r daily to the right side of the face of six adult animals until dosages of 4,200 r, 5,000 r, and 6,000 r were reached. While all animals did not react identically, the severity of the ensuing tissue reaction was usually proportional to the total dose, with

tissue damage restricted principally to the exposed side. The skin lesions of the cheeks of all animals healed completely after four months, leaving well-defined, moderately edematous, epilated, nonpigmented areas. Discoloration of the enamel of all teeth on the exposed side was produced in varying shades of brown in proportion to the dosage, a reaction also described as characteristically following severe oral irradiation in human beings. However, definitive caries has not developed in either the irradiated or nonirradiated teeth. Materia alba accumulated about the irradiated teeth in proportion to dosage. The buccal dental supporting tissues of the right side were severely damaged in most animals and partial or complete necrosis of the gingiva followed, exposing the roots of the teeth and necrotic bone. Irradiation of approximately 4,000 r produces early periodontal tissue damage in the monkey, but such irradiation, if applied to one area of the oral cavity, does not necessarily cause early tissue damage in an opposing area.

88. Cystogenesis in Odontogenic Epithelium of Syrian Hamsters Following Cephalic Irradiation. *Herman Medak and John S. Oartel, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* To study the effect of irradiation upon oral tissues, ten Syrian hamsters, whose bodies were shielded with 2 inches of lead, were exposed daily to 300 r to each side of the head, up to a total of 4,800 r for each animal. For irradiation 250 KVP G.E. Maxitron was used, operated at 250 kv., 30 Ma, 35 cm. std., and without external filtration. Six of the animals died within fourteen days after terminal irradiation, and the remaining four were sacrificed after four, five, six, and seven weeks. Varying degrees of keratinization were found in the altered odontogenic epithelium of all surviving animals, and a cyst developed in the base of an incisor of one animal. Sections of the incisors revealed liquefaction necrosis of the basal area of the pulp, followed by the formation of a cystlike cavity and cessation of growth in length. The enlarging cystlike cavity displaced and damaged the odontogenic epithelium, which continued to proliferate and was converted into a low cuboidal or squamous type of cells. In some areas the less differentiated odontogenic epithelium of all the hamsters produced varying degrees of keratinization and a large cyst was found in the base of one incisor. The wall of this cyst consisted of several layers of squamous epithelium with a center filled with strands of keratin. Regardless of the factors which produced this change, it is of considerable interest to note that the histodifferentiation of the odontogenic epithelium can be altered experimentally. A similar process of dedifferentiation may operate in the production of acanthomatous ameloblastomas.

89. Phosphamidase Content in Normal Tissues and Lesions of the Oral Cavity. *Julia Meyer and Joseph P. Weinmann, Division of Oral Pathology, University of Illinois, College of Dentistry, Chicago, Ill.* The phosphamidase content has been determined in thirty-seven oral biopsies, using the histochemical technic proposed by Gomori (*Proc. Soc. Exper. Biol. & Med.* **69**: 407, 1948). The following results were obtained: (1) The enzyme is absent in the normal human oral mucosa, the epithelial lining of the maxillary sinus, and the acinii and ducts of mucous glands. It is also absent in acute and chronic inflammation of the oral mucosa, and hyperkeratosis of the oral mucosa, provided that the lamina propria is free of inflammatory reaction. (2) Gomori's findings with respect to malignant neoplasms were confirmed. A high concentration of phosphamidase was found in all cases of carcinoma. The enzyme content tended to be higher in the less differentiated cases and

in the less differentiated elements of the tumors. (3) Nonmalignant lesions showing high phosphamidase content were the connective tissue of giant cell tumors and the epithelium of ameloblastoma, papilloma, and leukoplakia.

90. The Pulpal Response of the Rat Incisor to Cavity Preparation and Paraformaldehyde. *C. I. Mohammed and I. Schour, Department of Histology, University of Illinois, College of Dentistry, Chicago, Ill.* The effect of cavity preparation and filling with Aquadont (zinc oxide 2 parts, zinc sulfate pulverized 1 part and cornstarch 1 part) or Aquadont plus 1 per cent paraformaldehyde was studied histologically in the lower left incisors of fifty-seven white rats about 60 days of age. The animals were sacrificed from one to ninety-six hours after the operation. The unoperated right incisors of twenty-five of the experimental animals were used as controls. Eight of the experimental animals were given single injections of alizarin red S in order to measure the rate of dentin apposition. The odontoblasts showed functional and morphologic alterations. The mildest alteration consisted of a calciotraumatic line in the dentin which was the only change following the Aquadont alone. Following Aquadont plus 1 per cent paraformaldehyde, the morphologic changes seen in a varying number of odontoblasts ranged from reversible albuminoid degeneration to necrosis. Spikes of dentin matrix formed between the islands of functional odontoblasts in the advanced stages. The pulp reaction could be classified as a serous inflammation characterized by a dilation of blood vessels, serous exudate, and mobilization of histiocytes and fibroblasts. Polymorphonuclear leukocytes did not appear. These changes increased in intensity with the depth of the cavities. New young odontoblasts differentiated to replace those that were lost. In ninety-six hours, recovery was complete with Aquadont alone, partial with 1 per cent paraformaldehyde in shallow cavities, and absent with 1 per cent paraformaldehyde in deep cavities.

91. Tooth Mobility Measurements on Monkey Teeth. *H. R. Mühlemann, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Two thousand measurements of labiolingual tooth mobility were made with a dial indicator. Tooth mobility in 1/100 mm. varied according to the force applied. Plotting force against mobility gave characteristic curves which show two distinct phases—an initial steep, and a secondary gradually increasing phase. Periodontal structures were altered by post-mortem changes, heat, and formol fixation. Tooth mobility curves were constructed on the same teeth in vivo and at different post-mortem changes. A comparison of the tooth mobility curves for each tooth gives an indication of the mechanism of tooth mobility and the biophysical qualities of the periodontal membrane and bone. Thus, it was found that heat and formalin treatment destroy the initial phase of tooth mobility curves. The secondary phase is destroyed by formalin, but is only moderately altered by heat. Short interruption of the periodontal blood supply does not change the initial phase of tooth mobility curves. One to two hours after death the initial phase decreases by about 50 per cent; however, if multiple post-mortem measurements are performed, initial and secondary phases are two to four times as high.

92. Estimation of the Content of Calcium-Mobilizing Hormone in the Blood of the Rat. *Paul L. Munson, Alexander D. Kenny, Oscar A. Iseri, and Roy O. Greep, Biological Research Laboratories, Harvard School of Dental Medicine, Boston, Mass.* Within two hours after parathyroidectomy the serum calcium of 50- to 60-day-old rats, whether normal or calcium-depleted, falls 2 to 3 mg. per cent, thus indicating that the calcium-mobilizing hormone in the

circulation at the time of parathyroidectomy must be destroyed rapidly. The blood calcium continues to decline in calcium-depleted rats, leading to death in severe tetany between six and eighteen hours after parathyroidectomy. On the other hand, subcutaneous injection of 100 U.S.P. units of parathyroid extract per 100 Gm. body weight immediately after parathyroidectomy maintained the serum calcium at the preoperative level for at least eighteen hours. As little as 20 U.S.P. units of extract subcutaneously also fully maintained the calcium level of rats depleted of calcium four days for at least six hours after parathyroidectomy, while the same dose administered intravenously, although equally effective at two hours, was completely without effect at six hours. A subcutaneous dose of 5 U.S.P. units of extract had a detectable, but not completely protective, effect at six hours. It is concluded that in the rat, whether fed a diet adequate or deficient in calcium, the amount of parathyroid hormone normally in the circulation is relatively small, probably less than 5 U.S.P. units, and that the normal rate of secretion of the hormone may be as little as 2 to 3 U.S.P. units per hour. Experiments are in progress in which parathyroid extract is administered by continuous intravenous infusion in an attempt to clarify the problem further.

93. The Guinea Pig Mandibular Condyle Osteoclast Morphology and Some Preliminary Data on the Regularity of Their Occurrence. *Hugh I. Myers and Dale Rupert, University of Kansas City, School of Dentistry, Kansas City, Mo.* The mandibular condyles of one or two animals in the age groups 6, 8, 12, 16, 28, and 48 weeks were used. Ten micra staggered serial sections were made by mounting and staining every tenth section. Most of the cells were adjacent to bone or calcified cartilage. The size of the individual cells ranged upward to 80 microns for the long axis of elongated osteoclasts. They tend to be round in shape, but all shapes were found. Usually a considerable clear tissue-free area surrounded the osteoclasts, but in the marrow cavity they usually were not completely independent of the marrow tissue. The cell cytoplasm stained with an intensity equal to the bone matrix. The number of vesicular nuclei varied from one to ten or more. Although sufficient data have not been secured for statistical analysis, the data indicate these osteoclasts are a regular feature in growing animals and the number decreases with increasing age.

94. Procedures to Reduce Dental Function. *H. H. Neumann and N. A. DiSalvo, School of Dental and Oral Surgery of the Faculty of Medicine, Columbia University, New York, N. Y.* The present study has the limited purpose of comparing various methods of producing reduced dental function. These methods may serve in the investigation of the effects of abnormal function on the structure of the teeth. The following six procedures proved suitable in varying degrees: (1) The resection of the major masticatory muscles on one side; only moderately effective on account of rapid regeneration and because of hypertrophy of any nonresected strands. This method was used in one hog and one monkey with but fair results regarding unilateral mastication. (2) Intracranial motor nerve resection of the third branch of the trigeminal nerve produced good unilateral function in the monkey. As it is difficult to avoid concurrent resection of sensory nerves, evaluation of the major changes found in comparing the functioning and nonfunctioning half dentitions is a complex task. (3) Repeated injections of homologous serum into one temporomandibular joint in order to induce arthritis and thereby to impair function were used in ten rabbits. This method proved effective in the study of disuse effects of the

limbs in rabbits. Although arthritic changes were produced, insufficient interference with chewing was achieved and this approach seems of dubious value, at least in the rabbit. (4) Extraction of one quadrant of teeth, attempted in the monkey, appears to be more promising. These four methods allow for observing and confronting of the more and the less functioning half dentitions in the same animal. Two other procedures require control animals for comparisons, as they reduce function of the whole dentition: (5) Wiring of the jaws and feedings through a stomach fistula. (6) To evaluate the effect of an entirely soft or liquid diet administered for prolonged periods of time on the dental structure, we used forty Syrian hamsters in addition to previously described experiments on monkeys and on albino rats. Each of the mentioned procedures has its advantages and its objections. Different methods, if tried in parallel tests, offer the opportunity of verifying effects of nonfunction on the dental structure and of recognizing accidental by-products of one or the other of the mentioned methods.

95. Demineralization of Bone and Teeth by Organic Chelating Agents at Neutral pH's. *Gordon Nikiforuk, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Ontario.* The sodium salts of ethylenediaminetetraacetic acids are noncolloidal organic chelating agents that resemble the inorganic polyphosphates in their ability to form soluble nonionic chelates with a large number of metallic ions at neutral and alkaline pH's. This property forms the basis for a new and satisfactory method of demineralizing hard tissues without subjecting the specimens to low pH's. The sodium salt neutralized to a pH of 7.5 by hydrochloric acid, in a concentration of 0.5 M, at room temperature, makes a suitable demineralizing medium for small specimens (weighing about 0.5 to 1.0 Gm.). The use of higher temperatures greatly increases the speed of demineralization of bone specimens. The solution demineralizes small bone specimens at a rate about one-seventh that of acid solutions and about seven times as rapidly as a neutral decalcifying solution of magnesium citrate. This method should prove useful in histologic, histochemical, and chemical studies of bone tissues.

96. Cariostatic Effects of Ashed Foodstuffs Fed in the Diets of Hamsters. *Abraham E. Nizel, Tufts Dental School, Boston, Mass., and Robert S. Harris, Massachusetts Institute of Technology, Cambridge, Mass.* Previously it was reported that corn and milk produced in Texas caused the development of approximately 40 per cent less dental decay, when fed in the diet of hamsters, than corn and milk produced in northern New England. The present report relates to an experiment designed to determine whether the factor responsible for this caries difference was inorganic in nature. The ash (1,000° C.) from 100 pounds of Texas diet (A) and New England diet (B) was mixed into 100 pounds of each of these diets to produce ash-supplemented diets C and D, respectively, and the following four diets were fed to four groups of hamsters: From birth to autopsy (140 days) twenty-five hamsters were fed diet A or B,

		A	B	C	D
Corn	Texas	63		61	
	N. E.		63		61
Whole milk powder	Texas	30		29	
	N. E.		30		29
Alfalfa		6	6	6	6
Sodium chloride		1	1	1	1
Ash of diet A				3	
Ash of diet B					3

and twenty-one hamsters were fed diet C or D. Diets and distilled water were supplied ad libitum. In earlier investigations the hamsters were placed on the experimental diets at weaning. The results of the present experiment demonstrate that a more caries-susceptible tooth is produced in young hamsters when their mothers are placed on the test diets at the time the litters are cast. No significant difference in the caries rate was observed with diets A and B. This result is not in agreement with previous findings on different lots of corn and milk powder. The hamsters receiving the ash-supplemented diets C and D showed 60 to 70 per cent less carious teeth than those receiving unsupplemented diets A and B, and an even greater reduction in carious areas. Spectrographic analyses of single samples of milk from the two areas showed no significant difference in content of mineral elements. On the other hand, the corn sample produced in New England contained at least twice as much magnesium, copper, manganese, iron, aluminum, and molybdenum as the corn produced in Texas, and significantly less nickel.

97. Radiation Hazards From the Use of Dental X-ray Units. *W. E. Nolan and H. W. Patterson, Radiation Laboratory, Department of Physics, University of California, Berkeley, Calif.* This report endeavors to point out the radiation hazards involved with respect to the patient, the dentist, and the dental technician in the use of x-rays as a diagnostic tool. In many instances dentists and dental technicians are being unnecessarily exposed to x-rays. With respect to the patient undergoing oral roentgenography, it can be said that the dose rate can be as high as 280 r./min. Very definite hematological changes have been observed and are being thoroughly investigated. Recommendations have been suggested to eliminate overexposure, to both the patient and the personnel working in the dental office.

98. Development of Permanent Teeth. *Carmen M. Nolla, School of Dentistry, University of Michigan, Ann Arbor, Mich.* Serial annual radiographs of twenty-five boys (total radiographs 1,656) and of twenty-five girls (total radiographs 1,746) obtained from the files of the University of Michigan Elementary School were studied in order to develop a more accurate technic for the appraisal of the development of the permanent dentition as revealed by radiographs. The age period, on the average, extended for the girls from 55 to 194 months; for the boys from 68 to 201 months. The development of each tooth was appraised by studying the radiographs and graded on a scale from 0 to 10 (0, absence of crypt; 2, initial calcification; 3, one-third of the crown completed; 4, two-thirds of the crown completed; 5, almost completed crown; 6, completed crown; 7, one-third of the root completed; 8, two-thirds of the root completed; 9, almost three-thirds of the root completed, open apex; 10, apical end of root completed). Each set of observational appraisals was summarized individually, both graphically and numerically, thus providing material showing the dental development of each person and numerical values for the tabulation of dental age scales. Few developmental differences were shown between right and left teeth of the same kind. No significant sex differences in rate of development were observed; however, the girls started dental development at an earlier age. The general type of growth displayed by each tooth was the same; the equation which expresses the general type of rate change is an algebraic biquadratic ($y = ax^2 + bx + c$). Differences in the general sequence of development of teeth were not apparent between the sexes, with few exceptions. Individual differences of clinical significance are revealed by superimposing a graph of individual tooth growth on normative overlaps. The statistical summary of individual tooth development permits the preparation of detailed growth age scales which facilitate clinical application of the results.

99. In Vitro Changes in pH of the Dental Plaque and Surrounding Saliva. *William A. Nolte and Sumter S. Arnim, Dental Branch, University of Texas, Houston, Texas.* In vitro studies with glass electrodes reveal pH changes of dental plaque when dextrose is added. A drop in hydrogen ion concentration from pH 7.0 to pH 4.4 occurs quickly. When the electrode is surrounded by saliva of pH 7.0 the drop in plaque pH occurs in spite of the presence of the saliva. Tests of the surrounding saliva taken simultaneously with that of the plaque reveal no drop in salivary pH when 50 c.c. is used. If only 1 c.c. of saliva is used to surround the plaque, the pH of this saliva also drops with the plaque pH. Samples of saliva from around the plaque on teeth in the human mouth indicate a like drop occurs in the pH of mouth saliva adjacent the dental plaque to which dextrose has been added.

100. The Effect of Estrogen on the Supporting Structures of Molars in Rats and Mice. *A. G. Nutlay, S. N. Bhaskar, J. P. Weinmann, and A. M. Budy, Departments of Oral Pathology and Histology, University of Illinois College of Dentistry, and Department of Physiology, University of Chicago, Chicago, Ill.* This roentgenologic and histologic investigation deals with the effect of a-estradiol benzoate and diethylstilbestrol on the teeth, alveolar process, gingival epithelium, and epithelial attachment of groups of sixteen young rats, twenty-two young mice, and thirty-one 1-year-old mice. In newborn rats which received a total dosage of 0.05 to 1.30 mg. of a-estradiol benzoate over a period of from five to forty-two days, no changes could be seen. In the newborn mice which received from 0.80 to 1.30 mg. of diethylstilbestrol or a-estradiol benzoate over a period of from twenty-five to forty days there was a replacement of hemopoietic marrow of the alveolar process by fibrous marrow. Also the marrow spaces were reduced in size by new bone formation. In old mice which received injections or subcutaneous pellets of a-estradiol benzoate, there was an increased downgrowth of epithelial attachment along the root surface and into the bifurcation of roots. The gingival epithelium of the interdental papilla between first and second molars also proliferated in an almost tumorlike fashion. The interdental papilla lost its conical shape and became progressively blunted and concave. In final stages of the experiment there were deep interdental and interradicular pockets which then led to a marked resorption of the interdental and interradicular bony septa. The root surfaces showed many areas of resorption. The earliest of these changes were noted three weeks after the beginning of the experiment and they became progressively marked.

101. The Effect of Propyl Thiouracil on the Development of Molar Teeth of Rats. *K. J. Paynter, Division of Dental Research, Faculty of Dentistry, University of Toronto, Toronto, Ontario.* Since the administration of thiouracil and related compounds has been shown to inhibit thyroid hormone formation, producing much the same effect as surgical thyroidectomy but without the parathyroid disturbances often accompanying surgery, this method has been found useful for studying the effects of thyroid hormone deficiency on the development of structures such as the teeth in which calcification plays such a prominent role. Seven litters of rats of the Long-Evans strain were reduced in numbers to six animals per litter. Each litter of six was divided into two groups of three animals each, one of which served as the experimental group, the other as the control. Beginning the day after birth, each experimental animal received a daily subcutaneous injection of 6-n-propyl-2-thiouracil dissolved in 0.9 per cent saline. Amounts injected were as follows: 0.1 ml. from one to ten days; 0.2 ml. from ten to twenty days; and 0.4 ml. from twenty to twenty-five days. Control animals were injected with 0.9 per cent saline daily in equivalent amounts. Rats were sacrificed in pairs, an experimental animal and its litter-

mate control, at ages 5, 10, 15, 20, and 25 days. Two pairs were killed at each age. One-half of the mandible of each animal was dissected out for grenz ray study. The remainder of the head was fixed in Bouin's solution, decalcified, embedded in paraffin, sectioned, and stained in hematoxylin and erythrosin. Histologically, it was found that in the experimental group histodifferentiation, proliferation, deposition, calcification, and eruption all occurred in normal sequence at a rate commensurate with the general retardation in metabolism associated with hypothyroidism and at a rate slower than in the control animals. Grenz ray negatives of the mandible of each of the rats also demonstrated retardation in growth of the mandible and in tooth development and calcification in the propyl thiouracil-treated animals. There was also a marked increase in density of the cancellous bone of the mandible of the hypothyroid rats over that of the control group.

102. A Progress Report of Caries Studies Using the Artificial Mouth. *Ward Pigman, William Hawkins, Harold West, and Caroline Gaston, School of Dentistry and College of Medicine, Biochemistry and Bacteriology Departments, University of Alabama, Birmingham, Ala.* In the present work freshly extracted human teeth are mounted in plaster boxes and the surfaces are photographed at about eightfold magnification. The mounted teeth are placed in cylindrical glass funnels and a bacteriologic medium is allowed to flow dropwise over the individual teeth. The teeth are inoculated at weekly intervals by soaking for thirty minutes in a sample of pooled saliva. Usually, at regular intervals, the teeth are examined at thirtyfold magnification, and photographed when necessary. Under these conditions, lesions of the enamel have been produced which are very similar in appearance and position to natural carious lesions. By the use of bilaterally identical teeth, it has been shown that tooth surfaces brushed twice daily remain fairly sound for long periods, whereas unbrushed areas or roughened areas soften rapidly. Typical regions of attack of brushed teeth are occlusal grooves and pits, developmental pits and grooves, approximal surfaces, and cervical areas near the cemento-enamel junction. Individual teeth exhibit considerable difference in resistance to enamel attack. Apparently deciduous and unerupted teeth are much less resistant than old erupted teeth. There is some evidence for the development of a resistant film on erupted teeth. Enamel attack has been observed only when glucose is present in the media and streptococci and/or lactobacilli could develop. In the absence of glucose the teeth surfaces remain intact for long periods.

103. Particle Size in the Inorganic Portion of Teeth and Bone. *Aaron S. Posner, National Bureau of Standards, Washington, D. C.* A study of the particle size of teeth and bone by means of x-ray diffraction line-broadening and x-ray low angle-scattering is in progress. X-ray line-broadening results indicate crystallites of the order of magnitude of 300 to 600 angstroms are present. On the other hand, low angle-scattering technics show a particle size distribution ranging down to 50 angstroms. A knowledge of the particle size distribution in teeth may help explain the location of minor elements such as carbonate and fluoride.

104. The Value of the Radiographic Film in the Differential Diagnosis of Periapical Lesions. *Winlaw A. Priebe, Joseph P. Lazansky, and Arthur H. Wuehrmann, Departments of Oral Surgery and Radiology, Tufts College Dental School, Boston, Mass.* Single areas of chronic apical pathology as discovered in routine full-mouth radiographic film were removed by surgery and studied histopathologically, using serial sectioning technics. Previous to the surgery an additional adequate film of the area was taken and a radiographic

diagnosis of the lesion was made by four people, two of whom were teachers of oral surgery and two of whom were teachers of dental roentgenology. The lesions were classified according to the usual textbook descriptions into (1) abscess, (2) granuloma, and (3) cyst. In evaluating the results, the abscesses and granulomas were grouped under the single heading of granulomas. Histopathologically all lesions were classified as (1) cysts and (2) abscess-granulomas. The abscess-granuloma group included those showing inflammatory and degenerative changes without evidence of epithelial cells in lumen formation. Separate classification for abscesses and granulomas was impossible because of an inability in many cases to differentiate adequately between the abscess and granuloma. Lesions were evaluated as cysts if there was evidence of epithelial cells in lumen formation. In the 101 cases studied, 54.5 per cent were cysts and 45.5 per cent were of either the abscess or granulomatous type. No correlation could be established between radiographic interpretation and histopathologic diagnosis. Of the fifty-five cysts diagnosed by histopathologic examination only seven, or 12.7 per cent, were accurately interpreted radiographically by the four observers. The abscess-granuloma group was more successfully evaluated, but even in these cases only 58.7 per cent of the lesions were correctly diagnosed. This investigation suggests that the radiographs should be used only as a method of locating areas of apical change, and not as a means of differentiating between them.

105. Salivary Buffering Capacity Measured In Situ in Response to the Acid Stimulus Found in Some Common Beverages. *Robert H. Oster, Leah Miller Proutt, Burton R. Pollack, and E. Roderick Shipley, Department of Physiology, School of Dentistry, University of Maryland, Baltimore, Md.* This study of salivary pH was made in order to determine the buffering capacity of normal human saliva in response to a stimulant commonly found in the daily diet. Electronic pH measurement by a Beckman Model G meter with in situ electrodes makes it possible to obtain reproducible results under controlled conditions. Normal "resting" salivary pH readings were taken on 195 students and laboratory personnel. Approximately 3 ounces of a common carbonated beverage then was consumed, and pH readings, starting at 12 seconds after ingestion and continuing at 20-second intervals, were taken until the pH returned to a value equivalent to, or more alkaline than, the average normal reading. There were 385 experiments, averaging 10 pH readings per run, on ten carbonated beverages (average pH 3.0), a simulated beverage (pH 7.0), and a water control (pH 7.2). The range of "resting" values was 5.73 to 6.15, normally distributed, with an average of 5.97, standard deviation 0.40, standard error 0.022. The pH shift toward alkalinity, taken as buffering capacity, showed a significant difference in the slope of the curve over the 2-minute period. The average curve of increasing alkalinity versus time on ten test beverages showed a difference from the control curve of more than four times the standard error. Any difference greater than two times the S. E. indicates a significant change. The short time required for this buffering control after ingesting acid beverages is surprising; even more impressive is the fact that more than 50 per cent of the 385 runs showed this positive buffering effect in 12 seconds.

106. Growth Pattern of the Pig Mandible: A Roentgenographic Study Using Metallic Implants. *Irwin B. Robinson and Bernard G. Sarnat, University of Illinois, College of Dentistry, Department of Oral and Maxillofacial Surgery, Chicago, Ill.* A combined method of serial roentgenography and metallic implants was used to study the growth pattern of the mandible from 8 to 20 weeks of age in 9 litter-mate pigs. Amalgam implants were inserted into

prepared cavities in the mandible in the following areas: two implants close to the posterior border of the ramus, one in the center of the ramus, and one in the mental area of the body of the mandible. The metallic implants served as radiopaque guides for superpositioning roentgenographic tracings and also as points of reference in making various measurements. Lateral roentgenograms were taken immediately after surgery and at four-week intervals to study the rate and direction of growth. The distances between amalgam implants and between implants and certain borders of the mandible were measured. The distance between implants remained the same in every animal. There was an increase in the distance between the implants and all the borders of the mandible except the anterior border of the ramus. The rate and total amount of appositional growth was greatest at the posterior border and was approximately 2.5 times the amount of resorption at the anterior border of the ramus, thus resulting in a widening of the ramus. The mandible increased in height and length because of appositional growth at all borders except the anterior one of the ramus. The total height of the ramus was due to growth at the condyle (approximately 70 per cent) and at the inferior border (approximately 30 per cent). There was no evidence of interstitial growth. Gross measurements taken on the dissected mandible were in accordance with those taken on the final roentgenogram.

107. The Incidence of Unrecognized Diabetes in Dental Practice. *Rene Rochon and Joan Moran, University of Detroit School of Dentistry, Detroit, Mich.* It has been reported that more than 500,000 persons in the United States are known to have diabetes, but there are an estimated 2,500,000 who have the disease and do not know it, or will have it within a few years. Many of those potential diabetics visit dental offices. The question is, "How many?" To provide a possible answer a screening test of all the patients examined in the University of Detroit adult clinic was instituted. Nine hundred twenty-one patients of both sexes and of ages ranging from 12 to 80 were given a urinalysis examination. Eleven patients showed signs of the disease. Only one was aware of it. Thirty-two potential cases of nephritis also were discovered. The study will be continued.

108. Lactobacilli, Streptococci, and Dental Caries in the Hamster. *Morrison Rogosa and Murray Disraely, National Institute of Dental Research, Bethesda, Md., and Erling Johansen, University of Rochester, Rochester, N. Y.* Hamsters were isolated in individual cages. By means of the Johansen technic, caries was observed after thirty days and was present in 93 per cent of animals on Diet I after eight months, with a mean score of 51 and a high score of 168. Caries was generally absent on Diet II (similar to Diet I, but without sucrose). When present it was minimal or questionable at an incidence of 16.5 per cent, with a high score of 0.7 and a mean of 0.05. Quantitative salivary samples were obtained repeatedly by a new technic. The mean streptococcal count of ten runs was comparable on both diets. The mean lactobacillus count on Diet I was 1.9 times that on Diet II. However, there was great individual fluctuation. Often caries-free lactobacillus counts were manyfold caries counts. Hamster oral lactobacilli were greatly different from human types. Lactobacilli were the predominating fecal organisms in hamsters on both diets and the fecal lactobacilli apparently were identical with oral strains. One fecal pellet may contribute 100,000 or more lactobacilli to the mouth. Streptococci, lactobacilli, and gram-negative intestinal bacteria were more abundant in the feces of animals fed sucrose. No definite relationship was found between the oral bacteria studied and dental caries in the hamster.

109. An Approach to the Study of Interactive Phenomena Among Pairs of Indigenous Microorganisms. *Theodor Rosebury and David Gale, School of Dentistry, Washington University, St. Louis, Mo.* Evidence is accumulating that many of the effects exerted on the host by indigenous microorganisms depend on combinations of microbic species, hence on aspects of microbic ecology. Previous studies of this subject have dealt principally either with host factors or with individual microbic species or selected combinations. A method has been developed by us which may permit a broad survey of observable interactive phenomena occurring between pairs of microorganisms cultivable on suitable solid media. Sixteen pairs of overlapping spot cultures are arranged on one plate as a cross titration of four concentrations of each of two species. Counts of drop concentrations by adaptation of the method of Miles and Misra (*J. Hyg.* **38**: 732, 1938) permit estimation of limiting concentration-ratios for observed effects. Phenomena thus far observed include inhibition of growth of certain aërobic bacteria by many alpha and gamma streptococci (presumably the peroxide-catalase or "inhibin" effect of other workers); the inactivity in this reaction of three strains of alpha streptococci isolated from guinea pig fusospirochetal exudates; inhibition of growth of *Neisseria catarrhalis* by beta as well as alpha streptococci; and an apparent nondiffusible inhibitory effect of *Escherichia coli* on *Candida albicans*.

110. Microbiologic Differences in the Oral Cavities of Hunt-Hoppert Caries-Resistant and Caries-Susceptible Rats. *Samuel Rosen, Department of Zoology, Michigan State College, East Lansing, Mich.* The microbiologic flora of the mouths of numerous caries-resistant and caries-susceptible rats developed by Hunt and Hoppert were determined. Some rats were examined at weekly intervals beginning at 2 weeks of age. Determinations were made for lactobacilli, streptococci, yeasts, and actinomycetes. Distinct differences were noted between the resistant and susceptible animals with respect to lactobacilli and streptococci. Greater numbers of lactobacilli consistently appeared in the susceptible rats. *Streptococcus salivarius* was found in the susceptible animals more frequently than in the resistant animals. A streptococcus, which appeared as a rough, craterform colony on mitis-salivarius agar was found in all of the resistant rats which were examined, and in 18 per cent of the susceptible rats. It was noted that these susceptible animals were closely related. This streptococcus does not appear to be an important caries-inhibiting agent of the resistant rats, since it was acidogenic and showed only slight antibiotic activity against some strains of lactobacilli and *S. salivarius*. However, since this organism showed a tendency to occur in entire families, or else was not present, it is suggested that heredity may influence the types of microorganisms present in the mouths of rats and perhaps other animals.

111. Tissue Response to Tooth Movement in Normal and Abnormal Metabolic States. *Julian M. Rothblatt and Charles M. Waldo, Harvard School of Dental Medicine, Boston, Mass.* A method has been devised for the study of tissue response incident to tooth movement in laboratory rats. The jaws of normal rats subjected to placement of a rubber band between the molar teeth show, after three days, histologic evidence of remodeling with bone resorption and deposition in regions of pressure and tension. A typical, but minimal, response is evident as early as twenty-four hours after placement of the elastic. After three days the tissue response is near maximum, but still uncomplicated by beginning adjustment to new tooth positions. The most intense effect was seen in the upper right experimental quadrant, where hemorrhage, compression of the periodontal membranes, and undermining rear resorption often resulted from

excessive stress on the first and second molar teeth. The intensity of the response was diminished around the maxillary third molar tooth, and was even less in the lower opposing quadrant, where occlusal adjustment had occurred. There were no demonstrable changes in the upper and lower left quadrants, thus providing control in each animal studied. The effects of varied metabolic states are being explored. Preliminary surveys have been made on gonadectomized rats, adrenalectomized rats, hypophysectomized rats, rats on vitamin A, vitamin D, and lysine-deficient diets, and rats fed toxic doses of alloxan, cortisone acetate, testosterone propionate, thyroid powder, and propyl thiouracil. The present report is concerned only with hematoxylin- and eosin-stained histologic sections. By this method, the most striking departures from normal were seen in hypophysectomized and lysine-deficient animals, where the process of osteoclastic resorption predominated. Stress produced unusually extreme loss of bone in lysine-deficient animals, in which large numbers of osteoclasts had completely replaced normal bone.

112. Experimental Carcinogenesis in the Cheek Pouch of the Syrian Hamster. *John J. Salley, Division of Dental Research, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* A study to dtermine the susceptibility of the epithelium in the cheek pouch of the Syrian hamster to chemical carcinogenesis was carried out on thirty-six animals. The animals were divided into two series on the basis of the solvent used. Series A (acetone solvent) consisted of three groups: Group I—9, 10-dimethyl-1,2-benzanthracene; Group II—20-methylcholanthrene; Group III—3,4-benzpyrene. Series B (benzene solvent) was similarly divided into Groups IV, V, and VI utilizing the same compounds just enumerated, and in the same order. Each group consisted of six animals equally divided as to sex. A 0.5 per cent solution was used in each group and was painted on the epithelial surface of the pouch three times a week for sixteen weeks. Animals were observed for an additional nine weeks. Results on surviving animals were as follows: Group I—5 survivors, all with malignant tumors, onset seven weeks; Group II—five survivors, two with carcinoma, two benign, one negative, onset twenty-five weks; Group III—five survivors, four with carcinoma, one benign, onset sixteen weeks; Group IV—four survivors, three with carcinoma, one negative, onset eight weeks; Group V—three survivors, one with benign papilloma, two negative, onset twenty-five weeks; Group VI—four survivors, not tumors. The results indicate that the hamster pouch may serve as a useful tool in studying initiation, development, and metabolism of oral carcinoma because of its ready adaptability to in vivo microscopic observation.

113. Electrophoresis in Dentin. *Robert E. Sausen, James R. Jensen, Leon Singer, and W. J. Simon, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Experimental work was done on dogs' teeth to determine the limits and usefulness of electricity in accelerating ions through dentinal tubules. Solutions of radioactive calcium chloride were placed into shallow cavities and submitted to a direct current for varying amounts of time and current strength. The anode was located at the cavity surface, and the cathode on the cheek mucosa of the opposite side of the mouth. Upon completing a treatment, the cavity was filled with silver amalgam alloy and extracted immediately. Then the teeth were invested in clear acrylic resin in preparation for being ground to a thickness of 500 microns. Autoradiographs of these sections were used to obtain our results. When the circuit was closed, calcium ions moved through the dentin and eventually through the apex of the tooth. The path of movement was limited to those tubules involved in the cavity preparation. Penetration of the ions through dentin would not be complete unless the prod-

uct of time and current equaled a value of 0.5 milliampere minute. A maximum measurable concentration was obtained in 20 milliampere minutes. The concentration of ions began to increase first at the pulpal wall and later at the lateral borders of the path, from which it proceeded to saturate all of the involved dentin. Penetration of ions increased with the concentration of the solution. When no current was applied, diffusion of the ions required 10 minutes to reach the pulpal wall, and the concentration of these ions remained low in that time.

114. Study of Dental Caries in Children 1 to 6 Years of Age as Related to Socio-Economic Level and Food Habits. *Bhim Sen Savara and Theodore Suher, University of Oregon Dental School, Portland, Ore.* In 1950 we reported an investigation of incidence of dental caries in 1- to 6-year-old children from high and low socio-economic groups. Socio-economic status of the parents was deduced from the chararacteristics (e.g., whether public or private support, etc.) of the nursery attended by the child. This method of estimating socio-economic status according to nursery attended was criticized by sociologists as being poor in distinguishing socio-economic levels. At their suggestion, a questionnaire was designed to determine individually the socio-economic status of the parents of these same children, their food preferences, frequency of eating, and toothbrushing habits. Trained interviewers obtained home interviews from the parents of 279 of the 650 children. The remaining parents were not interested in cooperating or could not be contacted. Findings on the 279 children were analyzed by Chi-square and correlation technics. No significant association was found between dental caries incidence (def teeth) and (a) eating desserts after meals, (b) number of times a week cake, pie, ice cream, gelatin, cookies, or candy were consumed, (c) number of times a week fresh fruit was consumed, (d) frequency of food consumed between meals, (e) number of times child brushed teeth daily, and (f) income of parents. Statistically significant, though very slight, correlation was found between def teeth and (a) number of times a week canned fruit was consumed ($r = 0.23$, $P < .05$), (b) number of rooms per family ($r = 0.17$, $P < 0.5$), and (c) number of years of parental education ($r = -0.42$, $P < .05$). This reappraisal based on careful determination of socio-economic level of individual children thus revealed no practical correlation of dental caries with food and oral care habits or socio-economic status among children for which data were obtained. Our inability to estimate the bias introduced by the high nonresponse rate (57 per cent) leaves the results in doubt and further underscores the difficulties inherent in public survey methods of investigation.

115. Effects of Strontium and Fluoride on Bone Repair. *William B. Savchuck, National Institute of Dental Research, U.S.P.H.S. Hospital, Staten Island, N. Y.* Using the adult male rat as experimental animal, the influence of strontium and fluoride on the healing of the fractured left humerus was studied physically, chemically, and autoradiographically. *(1) Breaking Strength Series:* Sixty rats were classified according to the degree of overriding and alignment of the humeral fragments and distributed among four groups. Each group received drinking water of one of the following compositions five weeks after fracture: (a) 2mN fluoride (38 ppm), (b) 2mN strontium, (c) 2mN fluoride and strontium on alternate days, and (d) tap water. Rats were sacrificed twelve weeks after fracture and the humeri were tested for breaking strength on a specially devised assembly. Under the conditions of the experiment, bone repair, on the basis of mechanical strength, progressed quite independently of the influence of strontium or fluoride. *(2) Chemical Series:* A

total of twelve rats received alternate injections of Sr^{89} on four successive odd days, and NaCl or NaF on four successive even days during the fourth, eighth, or twelfth postfracture week. NaF had no effect on the deposition of Sr^{89} in the long bones. The percentage of Sr^{89} uptake in the fractured humerus was twice that in the normal one except for a drop in the twelfth week. *(3) Autoradiographic Series:* Following Sr^{89} injection two rats were sacrificed biweekly over a twelve-week period in order to study the progress of ossification in the fracture callus. Comparison of the autoradiographic and histologic findings shows that mineralization progresses dramatically in a centripetal direction toward the fracture gap.

116. The Rat Incisor Method for the Study of the Pulpal Response to Local Irritants and Stimuli. *Isaac Schour and C. I. Mohammed, Department of Histology, University of Illinois, College of Dentistry, Chicago, Ill.* Cavities were prepared and filled with various materials in the lower incisors of more than 100 rats. The animals were anesthetized by an intraperitoneal injection of Nembutal. The lower lip was retracted and an intraoral incision was made in the gingiva, exposing the alveolar bone below the mental foramen. Gingival hemorrhage was controlled with adrenalin. A cavity was prepared in the bone with the use of a No. 703 dental engine fissure bur, and was of sufficient depth to expose the lateral surface of the incisor. A second cavity about 0.75 mm. in diameter and 0.2 mm. deep was prepared within the dentin of the incisor with a No. 701 fissure bur and a No. 33½ inverted cone bur. The cavity was cleaned, dried, and filled with the substance under study. The bone cavity was filled separately. The operation was performed with a dissecting microscope. The lower incisor proved to be a useful test object for demonstrating the early effects of local mechanical and chemical injuries upon the odontoblasts and upon the pulpal tissue. The forming dentin and pulp serve as a kymographic record of local as well as systemic influences. This method is proposed as a biologic screening test for the effects of different chemicals upon the pulpal connective tissue.

117. Bacterial Factors in Nonspecific Gingivitis. *Stig Schultz-Haudt, Mary Ann Bruce, and Basil G. Bibby, Eastman Dental Dispensary, Rochester, N. Y.* Differential counts made on Gram stains regional bacterial smears from twenty-five patients with nonspecific gingivitis showed a large increase of fusiform bacteria, spirochetes, and Vibrios when compared with smears from clinically normal patients. The increase was greatest in the gingival crevice. In aspirations from gingival tissues and biopsy material, evidence of bacterial invasion of the deeper gingival tissues was found in one out of every ten specimens. Gingival debris was examined for products of bacterial activity. Hyaluronidase was found to be present in all cases of generalized chronic gingivitis and a separate factor, capable of causing a transient hyperemia in sensitized rabbit skin, also was demonstrated. Topical applications of hyaluronidase on human gingiva had an effect on the intercellular substance of the gingival epithelium. When the applications were repeated several times over a period of several days, openings in the epithelium similar to Stillman's clefts were observed in eight out of nine cases. Bacteria were observed invading these openings. Repeated applications over a period of from seven to eight hours to one and one-half days were found to cause an increase of Hotchkiss-staining polysaccharide material in the gingival epithelium.

118. Effect of Topical Application of Silver Nitrate on Dentin. *Solveig Schultz-Haudt, Richard G. Taylor, and Finn Brudevold, Eastman Dental Dispensary, Rochester, N. Y.* Carious dentin was removed from bilateral

proximal lesions in 284 deciduous molars, and the affected areas were made self-cleansing by slicing. Silver nitrate was applied unilaterally. Re-examination after one year revealed that silver nitrate had a marked caries-inhibiting effect. Recurrence of caries was observed on 18 per cent of the silver nitrate-treated surfaces, as compared with 78 per cent of the surfaces which received only mechanical treatment.

119. The Effect of Selective Desalivation of the Incidence of Dental Caries in the White Rat. *Abraham Schwartz and James H. Shaw, Harvard School of Dental Medicine, Boston, Mass.* Surgical removal of the major salivary glands (parotid, submaxillary, and major sublingual as well as the extra-orbital lacrimal glands) of white rats fed a cariogenic diet caused production of severe caries in eight to twelve weeks. To determine whether relative degrees of caries could be obtained by extirpation of single pairs of glands, or of combinations of pairs of glands, two experiments were designed in which such operations were performed on 200 white rats. Removal of the parotids or the submaxillary glands resulted in caries scores which were significantly greater than for the unoperated control animals, but which were significantly less than for the completely desalivated animals. Removal of the extraorbital lacrimal or the major sublingual glands resulted in no significant increase or barely significant increases in caries as compared to the control rats. Elimination of the major salivary glands on one side produced caries scores on that side significantly greater than on the intact side. The total caries score for rats from which the major salivary glands on one side were removed was significantly greater than that in the control animals, but significantly less than in completely desalivated rats. In groups of rats where combinations of two pairs of salivary glands were removed, the resulting caries scores were not uniformly greater than scores when only one of the two pairs was removed. Possibly this could be partially explained by significant increases in both the absolute gland weights and the ratio of the weight of the remaining glands to the body weight as compared to the controls.

120. Electron Microscopy of Carious Enamel and Dentin. *David B. Scott and John T. Albright, National Institute of Dental Research, Bethesda, Md.* Small pieces of carious enamel and dentin, selected so as to demonstrate early to advanced stages of the disease, have been dissected from human and rat teeth. All specimens have been fixed with neutral formalin, dehydrated with alcohol or Cellosolve, and embedded in plastic. Some specimens have been artificially demineralized with lactic, pyruvic, formic, phosphoric, or trichloroacetic acids; others have been soft enough to section without demineralization. Sections have been cut on a rotary microtome equipped with a thermal expansion adapter and glass knives. The location of microorganisms in both enamel and dentin has been studied. As yet, a definite orientation of organisms with respect to specific structures within the enamel has not been observed. In dentin, the sequence of bacterial invasion seems to be: filling of the dentinal fibers with organisms, followed by complete filling of the tubules, penetration from the tubules into the matrix, and finally complete disorganization of the matrix. Both rod and spherical forms of bacteria have been observed in enamel and dentin. Of special interest have been preparations made from enamel which, due to caries, has been sufficiently soft to be sectioned without artificial demineralization. In such sections a bacteria-free, yet softened, zone has been noted in advance of the region of bacterial invasion. In the hardest specimens which it has been possible to cut, the organic matrix has not been clearly visible in this zone, and a somewhat crystalline appearance has been noted; the presence of apatite

in the sections has been demonstrated by electron diffraction. However, the fact that typical organic matrix is actually present in such material has been established by the study of similar specimens which have been demineralized artificially. The present significance of such findings lies in the demonstration of a possible approach to determining whether the organic or the inorganic component of the enamel is damaged first in development of a carious lesion.

121. Microorganisms Antagonistic to Lactobacilli Predominate on Teeth of Caries-Free Animals. *Charles A. Scrivener, College of Physicians and Surgeons, School of Dentistry, San Francisco, Calif.* Scrapings from the teeth of caries-free dogs have been shown to contain microorganisms whose presence is antagonistic to the growth of lactobacilli. These determinations were recognized through the utilization of a double-pour-plate technic described at this meeting last year. Classification of the organisms is incomplete, although representatives from the various morphologic groups have been identified. Evidence is accumulating to indicate that bacterial antagonism may be a potent factor in dental caries prevention. Salivary calculus (under which caries does not progress) has been shown to contain certain lactobacillus antagonists, and similar antagonists have been isolated from many samples of human saliva. A method of utilizing bacterial antagonism for caries prevention has not been provided. However, indications are favorable for the application of this principle in the development of an effective preventive treatment for dental caries.

122. The Effect of Penicillin G Potassium Plus Calcium Carbonate Upon Surgically Exposed Dental Pulps of a Rhesus Monkey. *Alexander Seelig, Richard C. Fowler, and David Tanchester, Dental Department, Montefiore Hospital, New York, N. Y.* A histologic study to determine the reaction of the pulp to penicillin G potassium plus calcium carbonate was conducted on the teeth of one Rhesus Macaca monkey. The animal was anesthetized by an injection of Nembutal. Eighteen of the twenty existing teeth were used in this study, and these pulps were exposed. This was accomplished by drilling into the occlusal surfaces of the posterior teeth and the labial surfaces of the anterior teeth. The coronal portions of the pulps were macerated by the burr. No attempt at asepsis or antisepsis was made. In eleven teeth a thick paste of penicillin G potassium plus calcium carbonate was mixed with tap water and this was pushed into contact with the remaining pulp. Cement was then placed over this. Zinc oxide and eugenol were placed in four; Zinc oxyphosphate cement was placed in one; and two teeth were left unprotected. Seven days later the animal was sacrificed and the teeth were placed in formalin. H. and E. sections were made. A bridge of dentin was found in all of the teeth in which the penicillin and calcium carbonate had been placed. The pulps under this bridge were normal. There was evidence of a slight edema in some. The teeth in which no medicament had been placed, those which had been capped with zinc oxide and eugenol, and those which had been capped with cement showed various stages of tissue degeneration and abscess formation.

123. Growth of the Rabbit Snout After Extirpation of the Frontonasal Suture. *Abbe J. Selman and Bernard G. Sarnat, University of Illinois College of Dentistry, Department of Oral and Maxillofacial Surgery, Chicago, Ill.* The contribution of the frontonasal suture to growth of the snout was studied in fifty-four growing rabbits (twenty-nine experimental and twenty-five control) 6 to 12 weeks old. After surgically exposing and extirpating the suture either unilaterally (nine animals) or bilaterally (twenty animals), amalgam was inserted into prepared cavities in the frontal and nasal bones. The distances between implants, between implants and the extirpation margin, and the

width of the extirpation channel were measured. The measurements were repeated on the sacrificed animals two to twelve weeks later. Serial cephalometric roentgenographs were taken of twenty animals immediately after surgery and at fourteen-day intervals. From these roentgenographs the increased separation of the radiopaque implants was determined for specific periods, and this indicated that the rate of growth was declining. This was less rapid in animals with extirpations than in control animals. The gross findings revealed that: (1) Neither unilateral nor bilateral extirpation of the frontonasal suture affected longitudinal growth of the snout; (2) the anatomic suture did not reform; (3) where the frontonasal suture was extirpated bilaterally, the increment in distance between the frontal implant and the extirpation margin contributed about one-fourth, the extirpation channel about one-fourth, and between the extirpation margin and the nasal implant about one-half to the total longitudinal increment between implants; (4) in animals with unilateral extirpations, on the unextirpated side the increment in distance between frontal implant and frontonasal suture contributed about one-third, and between nasal implant and frontonasal suture about two-thirds to the total longitudinal growth.

124. The Relationship of the Salivary Glands, Endocrine System, and Dental Caries in the Albino Rat. *William G. Shafer and Joseph C. Muhler, Indiana University School of Dentistry, Indianapolis and Bloomington, Ind.* A study of the possible relationships of the salivary glands to the recognized endocrine system in the albino rat has been undertaken. The histologic structure of the submaxillary gland was found to be altered by gonadectomy of both male and female animals. Testosterone administered to males had an effect on the salivary glands opposite that of gonadectomy, while injection of diethylstilbestrol in females produced changes similar to gonadectomy. These changes were found to be correlated with the dental caries incidence. A continuation study in desalivated rats has shown marked atrophy of the uteri of female animals, but no detectable changes in the testes of desalivated males. In addition, definite histologic changes in the adrenal cortex of desalivated animals were noted. As was expected, the dental caries incidence was increased in the desalivated animals. A further study utilizing desalivated animals has shown that desalivated males breed poorly, whether mated with desalivated females or unoperated females, and that the average number of offspring per litter is small. In contrast, desalivated females mated with normal males have a high incidence of pregnancies with a larger number of offspring per litter. No changes have been noted in the histologic structure of the salivary glands of rats receiving maximal doses of cortisone for periods up to twenty-one days. Neither were there any changes noted in the salivary glands of rats which had been adrenalectomized and which survived for a period of approximately two months.

125. The Effect of Brushing the Teeth on Gingival Health. *I. L. Shklair, W. J. Carter, W. L. White, and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* Four companies of Naval recruits were selected and the gingival condition of each was determined by the method of Schour and Massler. Each individual was issued a new toothbrush and a dentifrice, along with instructions concerning their proper use. They then were required to brush their teeth with the dentifrice in the morning upon arising and in the evening before retiring for a period of five weeks, after which they were examined again and the PMA index was recorded. They then were issued another dentifrice and toothbrush, and were instructed to continue the oral hygiene procedures for another five weeks, at which time another PMA was recorded. During the first interval each of the men in the first company used Pepsodent tooth-

paste, while those in the second company used Listerine toothpaste. At the end of this period the men in the first company were issued Listerine toothpaste and those who had used Listerine toothpaste during the first interval used Pepsodent in the second interval. The men in the third and fourth companies used Colgate toothpaste and Ipana toothpaste, respectively, during the first interval and during the second interval the order was reversed. Thus, at the end of ten weeks it was possible to compare the over-all effect of brushing the teeth with a dentifrice and specifically to compare the Listerine with Pepsodent and to compare Colgate with Ipana on the same individuals. On the basis of the PMA initial examination, it was obvious that in general the new recruits did not ordinarily practice good oral hygiene procedures. During the first five-week period the gingival conditions improved immensely with the use of all dentifrices. Although there was a slight variation from dentifrice to dentifrice, it is questionable that the differences were significant. During the second interval the gingivae continued to improve, but the improvement was not so rapid. Here again the total change in all groups was greater than the differences between the groups. It was concluded that brushing the teeth with a dentifrice is highly beneficial to the gingival health. Although some dentifrices may be superior to others in regard to their effect on the gingivae, differences in the dentifrice are not as important as the procedure.

126. Bacteriostatic Action of Sodium Azide on Bacterial Types in Respect to Concentration and pH. *Marie S. Slawson and Marshall L. Snyder, University of Oregon Dental School, Portland, Ore.* The bacteriostatic action of sodium azide (NaN_3) in different concentrations at varying pH values (5.0, 5.5, 6.0, 6.5, and 7.0) of the medium, tomato juice peptone agar (TJA) was tested for a variety of bacteria: oral lactobacilli (twenty strains), Candida (twenty), Streptococcus (four), Staphylococcus (four), Bacillus (four), and miscellaneous gram-negative rods (eight). In general the inhibitory action of sodium azide was greater, with decreasing pH readings compared with their growth on the TJA without azide but with corresponding pH values. There was, however, a definite decrease of bacteriostatic behavior toward the lactobacilli at pH 6.0 compared with pH 5.5 or 6.5 at the 1:400, 1:800, 1:1600, and 1:3200 concentrations of sodium azide. To establish the limits of effectiveness of this chemical under conditions similar to those of routine lactobacillus counts, these bacteria were cultured on TJA adjusted to pH 5.0, 5.2, and 5.4 containing 1:10,000, 1:20,000, and 1:40,000 concentrations of sodium azide, respectively. Maximal action was obtained at pH 5.0 to 5.2 in a 1:10,000 concentration of azide. This level completely inhibited the development of any of these bacteria except the lactobacilli, all strains of which grew under these specified conditions.

127. Experimental Rat Caries. IV. Effect of a Natural Ash Mixture on the Caries-Conduciveness of an Otherwise Purified Diet. *Reidar F. Sognnaes and James H. Shaw, Harvard School of Dental Medicine, Boston, Mass.* This study was undertaken in order to determine whether the absence of caries in rats raised on a natural stock diet may be due to factors hidden in the mineral fraction of the stock diet. For this purpose the stock diet was ashed at 1040° F. (450° C.). The salt mixture so obtained was added to an otherwise purified, caries-producing diet in place of the synthetic salt mixture previously used. Litter-mate females were bred with litter-mate males under different dietary regimens: stock diet, purified diet, and natural ash diet. More caries was observed in litters raised on a purified ration exclusively than in those receiving the same ration with the natural salt mixture. The major reduction in caries was observed in animals that subsisted on the natural salt mixture ration

during development and eruption, as well as function, of the teeth. This difference appeared to be highly significant. It appears that the factors involved are most effective after prolonged ingestion by the mothers, as well as by the young litters before, during, and following eruption of their teeth into the environment of the mouth.

128. Histologic and Enzymatic Differences in the Submaxillary Glands of Normal and Hypophysectomized Male and Female White Rats. *Leo M. Sreebny, Department of Applied Materia Medica and Therapeutics and Division of Oral Pathology, College of Dentistry, University of Illinois, Chicago, Ill.* A proteolytic enzyme resembling trypsin was identified in the submaxillary glands of white rats in previous enzymatic studies. This enzyme was found to be specific for this gland. In the present report, the enzymic content and morphology of glands in normal and hypophysectomized male and female animals will be compared. Male and female Sprague-Dawley rats, 125 days old, were divided into three groups: (1) normal controls, (2) sham operated, and (3) hypophysectomized animals. Animals were sacrificed thirty days after operation, together with their normal controls, and the submaxillary glands were removed. A portion of the gland was fixed in formalin, stained with hematoxylin and eosin, and examined histologically. Another portion of the gland was frozen-dried. Determinations on extracts of the frozen-dried glands were done for alkaline protease activity, using Azocol and casein as substrates. The results may be summarized: (1) The submaxillary glands of normal male and female adult white rats, in contrast to those of mice, exhibit no morphologic differences; (2) the submaxillary glands of hypophysectomized male and female rats show a decrease in the dry weight of the gland, marked atrophic changes in the tubular portion, and a decrease in the intracytoplasmic granular content; (3) extracts of the submaxillary glands of normal or sham-operated male rats demonstrate greater alkaline protease activity than similar extracts from the glands of normal or sham-operated female rats of the same age; (4) hypophysectomy markedly reduces the proteolytic activity of extracts of the submaxillary glands of male and female rats.

129. Aphthous Stomatitis and Its Relationship to Herpes Simplex. *Marvin M. Stark, Harvard School of Dental Medicine, Boston, Mass.* Due to a lack of information dealing with recurrent aphthous stomatitis and its relationship to herpetic stomatitis, the complement fixation test (micro method), technic of Enders and Levens, is being utilized to determine the antibody titer of serum from patients with aphthous stomatitis to herpes antigen. The complement fixation technic affords a sensitive method for detecting herpetic antibody in human sera and is more economical of time and materials than neutralization tests. Sera were collected from thirty individuals as a means of establishing a base line for the procedure, and also to familiarize ourselves with the technic. These persons gave clinical histories of past and present herpetic infections. Complement fixing antibody titers ranging from 1:2 to 1:64 were present in almost all patients giving herpetic histories. Ten sera were collected from patients with aphthous stomatitis and eight gave significant titers to the herpes antigen. Investigations in the past have shown that 70 to 90 per cent of the adult population have circulating antibodies against the herpes virus. This study is being continued in order to investigate more adults with aphthous lesions who show no demonstrable titer to the herpes antigen. These patients will be followed during the course of their infection in an effort to detect any presence of antibodies or a rise in titer following infection, which is contrary to the pattern seen in herpetic infections. Herpes virus isolation also will be carried out on the aphthous patients to determine the relationship, if any, between herpes virus and aphthous lesions.

130. Contribution to the Histopathology of the Tongue. *George Stein and H. Gold, Columbia University School of Dental and Oral Surgery, The Dental Department of Montefiore Hospital, and the Neoplastic Service at Montefiore Hospital, New York, N. Y.* In order to establish a correlation between the nutritional state of a patient, the macroscopic observation, and the histologic findings of the tongue, clinical observations of the general nutritional conditions of a series of patients on the Neoplastic Service at Montefiore Hospital in New York were carried out. Particular emphasis was placed on the oral cavity, and especially the tongue. Routine laboratory tests, kodachrome photographs of the tongue, biopsies, and, when obtainable, post-mortem examinations of the tongue were performed. Some of the conditions observed were: (a) Changes in hornification, (b) shortening and fusion of filiform papillae, (c) "cobblestone" appearance of the tongue surface, (d) atrophy, (e) coating, (f) redness, and (g) dryness. The histologic background of these grossly observable changes is shown in photomicrographs of biopsies and post-mortem material, and a possible sequence of the pathology is discussed.

131. The Development of Various Types of Carious Lesions in Rats Fed on a Synthetic Diet. *Robert M. Stephan and M. Rachel Harris, National Institute of Dental Research, Bethesda, Md.* Two years ago the results of experiments were reported (*J. D. Res.* **30**: 484, 1951) showing the development of carious lesions on the gingival, proximal, and occlusal types in albino rats fed on a diet consisting of 66 per cent cane sugar, 32 per cent dry skim milk powder, and 2 per cent dried liver powder (Diet 580). This is a report on a series of experiments comparing the development of carious lesions on the above diet with lesions developing on a synthetic diet patterned after it. The synthetic diet consisted of 12 per cent vitamin-free casein, 83 per cent sucrose, 4 per cent H.M.W. salts, and 1 per cent of a mixture containing required vitamins (diet 593). In other experiments variations of Diets 580 and 593, in which the same supplements were added to both, were run in parallel so that the effects could be compared. The rats were Sprague Dawley Strain, 20 to 22 days old, and twenty-four experiments were run, with groups of twenty animals in each. Carious lesions were found which began on the buccal, lingual, proximal, and occlusal molar surfaces in rats fed Diet 593, as well as those on Diet 580. On both diets approximately one-half of the animals developed caries. Supplementing the diets with either 10 per cent casein, 4 per cent salts, or 3 per cent Wesson oil + 2 per cent cod liver oil gave a reduction of caries on Diet 580, and no caries at all on Diet 593. Supplementing with a mixture of B vitamins or with cod liver oil gave only a slight reduction in caries. Other variations in the diet produced comparable changes in both diets.

132. Effect of Beryllium Upon Acid Production by Lactobacillus Acidophilus. *Paul R. Thomassen, Henry M. Leicester, and William H. Schmidt, College of Physicians and Surgeons, San Francisco, Calif.* It has been known for some time that the production of caries can be markedly decreased by specifically poisoning the phosphatases essential in the fermentation of carbohydrates to lactic acid by acidogenic bacteria. This poisoning has been accomplished with fluorides and iodoacetic acid. It was undertaken in this laboratory to determine the effectiveness of Be as an inhibitor of acid production by *Lactobacillus acidophilus*. Be is an alkaline phosphatase inhibitor, and hence should have little effect upon acid production by this organism. To various concentrations of $BeCl_2$ in brain-heart infusion broth containing 1 per cent dextrose was added a drop of a twenty-four-hour culture of *Lactobacillus acidophilus*. After an incubation period of twenty-four hours at 37° C., the pH of each culture tube

was determined with a Coleman pH electrometer. Cultures containing 2.5×10^{-5} M and less of Be had no effect upon acid production. On the other hand, cultures containing 2.5×10^{-4} M and more of Be showed marked inhibition of acid production by *Lactobacillus acidophilus*.

133. Acid Formation in Human Saliva. *Paul E. Tullar, University of Texas Dental Branch, Houston, Texas.* Saliva samples were collected from caries-rich and caries-poor subjects, and exposed to various conditions of temperature, mixing, centrifugation, and dextrose concentration for periods up to six hours, during which pH measurements were taken on a Beckman pH meter. Unsugared saliva served as control and showed only a gradual increase in alkalinity and odor characteristic of proteolytic decomposition. Saliva containing 10 per cent dextrose dropped to as low at pH 3.8 in 30 to 60 minutes, beginning shortly after the addition of the dextrose. Saliva freed of its solid matter by centrifugation showed immediate cessation of further acid formation and remained at its achieved pH level. Inoculation of 10 per cent dextrose-aqueous solutions with the salivary centrifugate resulted in the same rapid acidification observed in the dextrose-saliva solutions. It is concluded from these observations that saliva carries enzymes capable of rapidly producing demineralizing acid concentrations in the presence of sufficient dextrose in both caries-rich and caries-poor individuals; that acidogenic enzymes are present in the suspended matter of saliva; and that saliva is not the good buffer previously thought, or that the acidifying potentialities of sugar in saliva are much greater than the work to date would indicate.

134. A Histochemical Evaluation of Gingival Healing on Adequate and Vitamin C. Deficient Diets. *Samuel S. Turesky and Irving Glickman, Tufts College Dental School, Boston, Mass.* Normal gingival healing, and gingival healing in acute Vitamin C deficiency were studied with the Lillie histochemical technic. In forty-four guinea pigs on adequate and Vitamin C deficient diets, the ground substance, basement membrane, and glycogen distribution in healing gingiva, following gingivectomy, were evaluated from biopsy specimens. In acute Vitamin C deficiency, the rate of gingival healing was retarded, surface inflammation persisted, and ground substance formation and maturation of basement membrane were retarded.

135. Titratable Acidity, Titratable Alkalinity, and pH of the Saliva for 315 Children Aged 5 to 11 Years. *Naomi C. Turner, James H. Scribner, and Jack T. Bell, Forsyth Dental Infirmary for Children, Boston, Mass.* Numerous studies have been reported in which pH and dental caries incidence are considered. Most of these studies indicate a lack of close relationship between pH of whole saliva and caries incidence. Fewer studies have been reported in which titratable acidity or titratable alkalinity and dental caries incidence were points of consideration. There is some uniformity in reporting an association between dental caries incidence and titratable alkalinity. This study of the saliva of 315 children, aged 5 to 11 years, reports titratable acidity (measured with 0.001N NaOH, phenolphthalein indicator); titratable alkalinity (measured with 0.001N HCl, methyl red indicator); pH with Beckman potentiometer using calomel and glass electrodes. The titratable acidities ranged from 0.4 to 10.4 with a mode of 2.7 and a mean of 3.01, σ 1.4; the titratable alkalinities ranged from 4.9 to 21.2 with a mode of 9.9 and a mean of 10 ml., σ 2.7; the pH range was 6.4 to 8, mode 7.3 and mean 7.32, with a σ of 0.46. The study indicates that low acidity and high alkalinity of the saliva are associated with caries-free individuals, while high acidity and low alkalinity tended to be found in the saliva of children with

a high caries incidence. When caries incidence was considered as DF (diseased and filled surfaces), DQ (dental quotient), or as classes of teeth with varying susceptibility as the criterion, "t" values at 0.01 significance levels were found more consistently for titratable alkalinity than for titratable acidity or pH. In connection with these studies our attention has been directed toward the amphoteric character of amino acids. The basic amino acids, lysine and arginine, might be investigated as a source of the higher alkalinities of individuals free from dental caries.

136. The Glucose Content of Carious Dentin. *J. F. Volker, University of Alabama School of Dentistry, Birmingham, Ala.* The glucose content of the carious dentin of ten clinical subjects with posterior bilateral tooth decay has been investigated. Two hours after eating, carious material has been removed from the posterior teeth on one side of the mouth and its glucose content determined by the Folin-Malmros method. Immediately thereafter, the experimental subject has washed his mouth with 50 c.c. of a 10 per cent glucose solution. Ten minutes later carious dentin has been removed from comparable posterior teeth on the opposite side of the mouth and analyzed for glucose. The glucose content of the carious dentin removed before the experimental procedure varied from 42 to 128 mg. per cent. The glucose content of the carious dentin excavated after the experimental procedure varied from 90 mg. per cent to 770 mg. per cent. In five of the subjects there was a marked increase in the glucose content of the carious dentin following the use of the mouthwash. The glucose content of the carious dentin in the remaining five subjects was unaffected by the experimental procedure.

137. The Permeability of Human Tooth Enamel, or A Quantitative Study of the Permeability of Human Teeth. *C. Wachtl and L. S. Fosdick, Northwestern University Dental School, Chicago, Ill.* There is some information to indicate that the morphologic characteristics of carious lesions in the enamel may be dependent upon the permeability of the tooth to ions and molecules. With this in view, the centripetal permeability of human teeth was investigated. Sound anterior human teeth were selected, and the pulp was removed manually. Each tooth was set up in such a way as to act as a membrane. The outside surface of the tooth was exposed to water solutions containing different concentrations of ammonia, urea, and urea in presence of either glucose or sucrose. Materials which permeated the tooth were determined quantitatively by chemical means at various time intervals. It was found that under conditions of the experiment human anteriors are permeable to ammonia and urea, but are not permeable to sucrose and glucose. In the presence of either glucose or sucrose the permeability of the tooth to urea decreases. This decrease can be partially explained by the creation of an osmotic pressure differential between the outside and the inside of the tooth, the direction of which is opposite to the diffusion gradient of the small molecules. There also is a competition for diffusion passages and an increased viscosity of the solution. There is an approximately linear relationship of amount diffused with time. A possible mechanism of the passage of urea through the tooth lattice is advanced. Variables influencing permeability, such as surface exposed, age of teeth, sex, differences among individuals, and differences among the teeth of a single individual, are discussed.

138. A New Method for the In Vitro Production of Artificial Calculus. *S. Wah Leung, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* A new method for the production of artificial calculus on extracted teeth has been devised. Previous methods subject the teeth to continuous submersion in sa-

liva or other media. Such a condition, however, is not normally found in the mouth. In their natural environment the teeth are not continuously submerged in a pool of saliva, but, instead, are subjected to exposure to air and partial drying for varying periods of time, as during speaking, breathing, and eating. These phases of exposure and drying may be important in facilitating or increasing the formation of calculus. A method for the in vitro production of artificial calculus, based on the principle of cyclic partial drying of the tooth surfaces, is described. This method has been successfully used for two years and has been found to yield results quantitatively superior to the method of continuous immersion. Analyses of the deposits show them to be similar to clinical calculus in organic and moisture content and in calcium and phosphorus concentrations. The present apparatus permits the simultaneous study of 60 individual saliva samples utilizing 120 teeth. This capacity may be changed to any number by very simple alterations in design. The method is well suited for studying, either singly or collectively, many of the factors believed to influence calculus formation. This method has been used for studying the effect on calculus formation of (1) adding different substances to the saliva, (2) pretreating the tooth surfaces with different compounds, and (3) alternating the bacterial flora of the saliva.

139. Calculus Formation From Saliva as Influenced by an Altered Bacterial Flora. *S. Wah Leung and Paul V. Morgan, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* The role of bacteria in the formation of calculus has been reinvestigated by the use of the in vitro calculus-producing method previously described. Specifically, comparisons were made of the calculus-producing properties of saliva which had been rendered bacteria-free with that of normal untreated saliva, and with saliva heavily inoculated with additional bacteria isolated from clinical calculus. Initial attempts to secure bacteria-free saliva by autoclaving showed that both autoclaved saliva and autoclaved saliva reinoculated with its own bacteria-contaminated material previously removed by centrifugation, produced no calculus. Chemical sterilization of the saliva with mercuric chloride, phenol, chlorothymol, propylene oxide, sodium fluoride, Aseptoform P and Aseptiform M were then tried. Mercuric chloride was found to maintain a degree of sterility satisfactory for our purpose. The effect on the calculus-producing ability of saliva, rendered sterile by mercuric chloride, was thus compared with saliva inoculated with heavy suspensions of dextrose-starch broth cultures of single types of organisms isolated from clinical calculus, and with normal untreated saliva. Three organisms, of several isolated from calculus, were selected for testing in this manner. The results showed that the addition of mercuric chloride to saliva did not inhibit, and the addition of organisms isolated from calculus did not enhance, calculus formation. It should be pointed out, however, that the organisms were not used in the numbers or proportions present in the natural calculus deposits.

140. Method for the Study of Tissue Response to Tooth Movement. *Charles M. Waldo, Harvard School of Dental Medicine, Boston, Mass.* Previous studies of tissue response to tooth movement in dogs, monkeys, and human beings have involved complicated technics and have been limited to small numbers of individuals, usually of unknown genetic background and metabolic status. A method has been devised using the laboratory rat, affording greatly increased possibilities for experimental control and the use of larger numbers of animals. Using a specially designed instrument, a segment of rubber band 0.8 mm. in cross section is inserted between the maxillary right first and second molars of 100 to 150 Gm. rats under ether anesthesia. Older rats were found to present

difficulties, because wear of the cusps produces sharp edges which cut the rubber. Definite separation is obtained in twenty-four hours, and maximal separation in three days. Rubber segments will remain in place for five to seven days, after which a majority are lost. Stresses exerted by the rubber also affect teeth of the opposing lower right quadrant. Left quadrants may be used as controls. To produce comparable sections, a precise orientation technic is required in histologic preparation. Mechanical interference brought about by this simple "orthodontic appliance" is reproducible with a high degree of standardization. Work is now in progress with this method to study the effects of metabolic factors on tissue response to tooth movement.

141. A Clinical Evaluation of Analgesic Drugs Used Following Minor Oral Surgery. *W. L. White, W. J. Carter, Dental Research Facility, and J. R. Seal, USN Naval Medical Research Unit No. 4, Great Lakes, Ill.* Because of the need for qualitative and quantitative clinical evaluation of analgesic drug action, two analgesic-antipyretic drug combinations have been studied with respect to their action when administered to patients for post-extraction pain. The study was limited to cases which had no more than four teeth extracted. Three types of plain white tablets (a 5 gr. aspirin, acetophenetidin, and caffeine combination; a 5 gr. N-acetyl p-aminophenol, and carbomal combination; and a placebo) were placed in coded envelopes and dispensed by the oral surgeons of five Naval dental clinics. The patient was instructed to take one tablet every four hours in case of postextraction pain. The data of 600 cases were subjected to biostatistical review, and a chi square determination proved the significance of the sampling. The results disclosed a significant difference in the action of the drugs; the aspirin, acetophenetidin, and caffeine combination produced analgesia in a greater percentage of cases. Pain threshold changes are also being studied and will be discussed with other supplementary data.

142. A Preliminary Report on the Quantitative Interrelationships of Microorganisms Cultivable From Human Saliva. *Ned B. Williams, Charles F. Eickenberg, and Beverly M. Florey, Department of Microbiology, School of Dentistry, University of Pennsylvania, Philadelphia, Pa.* A recent study on the cultivability of various kinds of microorganisms in human saliva indicated that certain ones could be cultivated in high frequency from a number of the human beings tested. This work also indicated that it would be possible to make quantitative estimates of these various organisms per unit volume of stimulated saliva. This report is concerned with the methods employed and preliminary findings. Samples of saliva were collected by paraffin stimulation before breakfast; study was begun at least two hours after collection. The saliva was diluted and divided into two aliquots; one was diluted serially for immediate inoculation of culture media, and the other was exposed to high frequency vibration for various time intervals before serial dilution and inoculation of culture media. Nine different culture media, mostly selective, were inoculated simultaneously. Selective media were inoculated to obtain colony counts of *Streptococcus salivarius,* lactobacilli, fusiforms, staphylococci, yeasts, an unidentified gram-negative diplococcus, various kinds of streptococci, and for various kinds of gram-negative bacteria. A culture medium, not considered selective, was used for total colony counts of oral bacteria. Aerobic incubation was employed for all media, except that for fusiform bacteria, at 37° C. Preliminary results indicate that there are marked differences in the relative numbers of colonies of these various organisms when counts from the saliva of children and adults are compared. Counts vary with different saliva samples from the same persons on different days, and between

samples from different persons. However, the quantitative interrelationships of the various microorganisms appear to be characteristic for each person. The results indicate that the method will be useful in studying the microbial ecology of the oral cavity.

143. The Effect of Nonanatomic Geometric Occlusal Carving on Chewing Efficiency. *A. Yurkstas and C. W. Sauser, Tufts College Dental School, Boston, Mass.* In an effort to evaluate chewing efficiency in the flat plane type of occlusion, various combinations of geometric patterns were carved into specially constructed appliances. Peanuts, carrots, and ham were used as test foods. Five subjects participated in this study, and a total of approximately 950 chewing tests were performed. The results of the testing program indicated that: Performance was greater when both upper and lower occlusal surfaces were provided with cutting edges, regardless of what direction or shape they presented. Greatest performance resulted from the use of cutting edges which ran a mesiodistal course in the area of occlusion, regardless of what opposed them on the lower bite plane. A correlation of 0.56 was found between peanuts and carrots, 0.66 between carrots and ham, and 0.71 between peanuts and ham. This indicated that occlusal carvings did not act similarly on different types of foods. In an effort to determine which type of pattern would perform exceptionally well on all foods, it was found that a pattern which included longitudinal markings on both upper and lower biting surfaces was most efficient with carrots, peanuts, and ham. Attempts to relate performance with occlusal contact area resulted in inconclusive findings.

144. Healing of Periodontal Pockets After Curettage. *Helmut A. Zander and Erwin M. Schaffer, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Five periodontal pockets were curetted. Five weeks later the teeth and adjoining periodontal tissues were removed. The tooth-tissue blocks were decalcified, embedded in celloidin, and sectioned serially for microscopic study. Photomicrographs of these sections show deposition of osteoid tissue on the curetted root surface, a new periodontal membrane, addition of new bone to the old alveolar bone, presence of cement and dentin particles in the connective tissue, and osteoid tissue forming around these particles.

145. Deposition of Fluorine in the Bones and Teeth of Rachitic Rats. *I. Zipkin, R. C. Likins, F. J. McClure, National Institute of Dental Research, Bethesda, Md.* Twenty weanling female Sprague-Dawley rats received the low phosphorus rachitogenic diet of Coleman, Becks, and associates (0.018 per cent P, Ca/P = 24.6). Litter-mated rats were pair-fed with the same diet, supplemented with phosphorus to prevent rickets (0.56 per cent P, Ca/P = 0.79). All rats drank a 20 ppm F solution; the fluid consumption was measured and the total fluoride intake was equalized. All rats were roentgenographed at the end of six weeks. The femurs, mandibles, molars, and incisors were analyzed for fluorine. The femurs and mandibles of the pair fed rachitic rats showed a 63 per cent and 47 per cent reduction, respectively, in the percentage of ash, but a 59 per cent and 61 per cent increase, respectively, in the percentage of fluorine of the ash when compared to the nonrachitic pair-fed rats. Similarly, differences were observed for the molars and incisors, but to a much lesser degree. The total fluorine in milligrams deposited in the femurs was 0.183 and 0.372 for the pair-fed rachitic and nonrachitic rats, respectively; for the mandibles, 0.114 and 0.193; for the molar teeth, 0.056 and 0.050, respectively; and for the incisors

0.114 and 0.113, respectively. The percentage of fluorine intake retained by the femurs was 1.48 and 3.16 for the pair-fed rachitic and nonrachitic rats, respectively; for the mandibles, 0.93 and 1.64; for the molar teeth, 0.46 and 0.42, respectively; and for the incisors, 0.94 and 0.96, respectively.

V. Abstracts of Papers Read by Title

146. Experimental Oophorectomy: Possible Influences Upon Alveolar Bone. *R. Gordon Agnew, H. Henry Weisengreen, and Russell D. Coleman, University of California, College of Dentistry, San Francisco, Calif.* It has been suspected from time to time that ovarian dysfunction might be a factor in the production of the periodontal disease complex commonly termed periodontosis. This viewpoint not only pertains to the gingival and mucosal aspects which have been investigated to some degree, but also to bone alterations, regarding which very little experimental evidence is available. This report, which is preliminary in nature, deals with one aspect of a long-term study of the effects of endocrine deprivation upon the gingival and periodontal tissues, specifically investigation of possible roentgenographic diagnostic signs indicative or suggestive of bone changes attributable to ovarian dysfunction. The Rhesus monkey is used. To date eight animals, approximately 4 to 8 years of age, have undergone bilateral oophorectomy. Surviving animals have been x-rayed at intervals up to a period of 18 months. The roentgenographic bone picture to date has not indicated structural changes of clinical usefulness in diagnosis of ovarian deprivation. The trabecular structure, circumradicular lamina dura, the periodontal membrane space, and the interdental crest bone have not shown alterations of dimension, pattern, or density beyond expected tolerance limits.

147. Hormonal Control of Growth and Eruption of Upper Rat Incisor. I. The Effect of Thyroidectomy at Birth and the Response to Growth Hormone and Thyroxin. *Louis J. Baume, and Hermann Becks, College of Dentistry and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* One normal control and four experimental groups of 63-day-old rats of the Long-Evans strain were studied. Experimental rats were thyroidectomized at birth; one group was retained as operated control; another operated group was injected daily with 0.5 mg. purified pituitary growth hormone intraperitoneally from their thirty-second to sixty-third days of life; an equal group received 2.5 μg of dissolved thyroxin subcutaneously for thirty-two days, and the last group was given both dosages in combination. Beginning with the thirty-second day, a marking was made weekly with a jeweler's file at the gingival margin of the labial surface of the upper incisor. The distance between two lines thus represented the eruption rate for a seven-day period. After the animals were sacrificed (sixty-third day of life), the heads were split in half, roentgenographed to record the size of the upper incisor as represented by the radius of the outer curvature, and histologic sections of the upper incisor were prepared. The normal control groups showed an average weekly eruption rate of 2.8 mm. and a radius of curvature of 5.9 mm. Thyroidectomy engendered a 45 per cent reduction in the eruption rate to 1.5 mm. and a 25 per cent decrease in the radius to 4.7 mm. Histologically, a stunting in the proliferation and a displacement of the odontogenic epithelium, together with a marked delay in epithelial histodifferentiation, were noticed. All tissues exhibited decreased vascularization. Growth hormone therapy elicited a 10 per cent increase in the eruption rate to 1.9 mm. and a 7 per cent increase in the radius to 4.9 mm. over the thyroidectomized controls. His-

tologically, there was a definite activation of the mesenchymal components of dental tissue without an activation of epithelial histodifferentiation. Thyroxin accelerated the eruption rate by 27 per cent to 2.4 mm. and increased the radius 17 per cent to 5.5 mm. of incisors of thyroidectomized rats. Histologically, restored activity of odontogenic epithelium, proliferation as well as histodifferentiation, is evidenced. All tissues showed increased vascularity. Nevertheless, some enamel hypoplasias occurred. Combination therapy gave optimal results; eruption rate was increased by 28 per cent and the radius by 21 per cent. The histologic aspect of newly formed dental tissues was as regular as that of intact rats.

148. Hormonal Control of Growth and Eruption of Upper Rat Incisor. II. The Effect of Hypophysectomy Following Progressively Longer Intervals. *Louis J. Baume, Hermann Becks, and John C. Ray, College of Dentistry and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, Calif.* The material comprised three groups of female rats of the Long-Evans strain. Group I, consisting of seventy intact rats, represented normal controls: Sixty of them were sacrificed in groups of three to six with advancing age in order to ascertain the age changes in size and histology of the incisors. In the remaining ten rats, the eruption rate was measured weekly for 51 weeks (357 days). Group II consisted of seventy rats which were hypophysectomized at 28 days of age: Sixty of them were sacrificed for metric and histologic purposes after progressively longer postoperative intervals which matched the ages of normal controls. Nine operated rats of this group were kept alive for 51 postoperative weeks (357 days), during which the eruption rate was recorded. Group III was represented by ten hypophysectomized rats which were sacrificed after long postoperative intervals (332 to 444 days). The eruption rate of the incisors was recorded during their last 7 weeks of life. The radius of curvature of the upper incisors of normal rats between 35 and 120 days of life showed rapid increase by 2 mm. to reach an average of 6.9 mm. It gained an additional 0.5 mm. during the following period (120 to 357 days of age). The weekly eruption rate exhibited a periodic variation and a decreasing gradient with increasing age. During 329 days it averaged 2.51 mm. ($\pm$0.13), the maximum value of 2.8 mm. ($\pm$0.17) occurring at 63 days of age when a slow decrease set in. Hypophysectomy engendered a stunting of apical growth which became noticeable metrically and histologically in the third week after operation. Dentinogenesis at pulpal surfaces, however, proceeded continuously effecting a final obliteration of the pulp canal. During a postoperative interval of 47 weeks the radius of curvature increased an average of only 0.3 mm. The weekly eruption rate dropped immediately after hypophysectomy to about one-half the normal amount to arrive at a minimum level of 0.24 mm. after 21 weeks. The great range of variation in this old-age group (0.7 mm. to 0.08 mm.) depended upon the absence or presence of apical folding. Individual analysis showed that the severely folded incisors found in 40 per cent of the old hypophysectomized rats virtually ceased erupting and increasing in radius, while unfolded incisors maintained minimal growth and eruption rate. The folding phenomenon showed the following histopathologic sequence: (1) Agenesis or atrophy of inner enamel epithelium; (2) disturbed calcification of the adjacent mantle dentin, and (3) impaired induction and aplasia of odontoblasts.

149. Hormonal Control of Growth and Eruption of Upper Rat Incisor. III. The Response to Growth Hormone and Thyroxin and the Combination of Both Following Hypophysectomy. *Louis J. Baume, and Hermann Becks, College of Dentistry, and the George Williams Hooper Foundation for Medical*

Research, University of California, San Francisco, Calif. Ninety rats of the Long-Evans strain were divided into three groups: Group I consisted of young female hypophysectomized rats (operated at 28 days) which for 437 days received either 0.5 mg. growth hormone or 5 γ thyroxin, beginning twelve days after operation. Group II consisted of old hypophysectomized rats (operated at 28 days) which after a postoperation interval of 229 to 470 days received for seven weeks one of the following daily dosages: (a) 0.2 mg. growth hormone, (b) 5 γ thyroxin, or (c) the combination of both hormones. Group III was composed of intact, 207-day-old rats which received during 436 days an increasing dose of growth hormone (average 1.7 μg). Growth hormone in Group I did not influence the eruption rate, which remained equal to the hypophysectomized controls. It effected an increase in size of the incisors to normal average and the development of maxillary prognathism. Histologically, a stimulation of the mesenchymal tooth components prevented folding, but not atrophy, of the enamel epithelium. Growth hormone in Group II did not accelerate the eruption rate either; in the folded incisors it elicited some increase in size, while in severely folded ones it failed to reorganize the odontogenic tissues. In the intact adults it produced oversized incisors without enhancing their eruption rate. Thyroxin in Groups I and II increased the eruption rate 36 per cent and 17 per cent, respectively, and the size of the incisors to a lesser degree. Histologically, a stimulation of epithelial proliferation and histodifferentiation was evident. With combination therapy, metrically and histologically optimal results were obtained. Even in severely folded incisors an almost complete reorganization of the atrophied enamel organ occurred at the apical end. It is concluded that tooth eruption requires the endocrine stimulation of two hormones. Growth hormone presides over the basic process of the proliferation of the mesenchymal tooth components. Thyroid hormone presides over the maturation process by controlling the activity of the odontogenic epithelium which induces differential growth of the dental and predental tissues.

150. Evaluation of the Micro-Antimony Electrode and Preliminary Intraoral pH Measurements. *Finn Brudevold and F. Clarence Thompson, Eastman Dental Dispensary, Rochester, N. Y.* The micro-antimony and glass electrodes gave similar pH readings in a number of organic acids, but the accuracy of the antimony electrode was affected by hydroxyl acids, oxidizing and reducing agents. Lactic acid and thioglycolic acid (reducing agent) in concentrations exceeding 0.001 molar gave higher readings with the micro-antimony electrode. Peroxide in concentrations exceeding 0.0001 molar depressed the antimony electrode reading. On the basis of laboratory observations and comparative readings with the glass electrode in the mouth, it was concluded that the micro-antimony electrode was suitable for measuring gross pH changes in the mouth. Measurements of different areas on the same tooth frequently showed a marked variation in pH of plaque material. A pH as high as 8.9 was found in heavy deposits on teeth and also on mucous membrane. The pH in periodontal pockets was in the range 7.4 to 7.6, while carious lesions generally were on the acid side. Intraoral pH measurements in the hamster were higher than those obtained in man, but a similar fall in pH, as occurs on human teeth, was observed after sugar application.

151. The Oxygen Uptake of the Gingiva. *Martin Cattoni, School of Dentistry, University of Texas, Houston, Texas.* The oxygen uptake of the soft tissue involved in periodontal pockets was measured, using the Warburg manometric technic. The subjects chosen for this study were patients with various degrees of periodontal disturbances. Full-mouth radiographs and a complete case history of each patient were taken. In addition to the clinical picture,

these results were compared with the histologic picture. The medium used was Ringer phosphate solution (pH 7.5). The value of oxygen consumption was calculated in microliters, per square millimeter, per hour. This value is the QO_2. One piece of the tissue was saved for histologic study, and normal gingivae were taken for comparison with the pathologic tissues. The criterion for the normality of the tissue, besides the histologic examination, was a careful clinical study. In the histologic picture there were small variations of a uniform pattern. There is no correlation between the depth of the pocket and the histologic picture. Studying the oxygen uptake, in chronic, proliferative changes, the QO_2 increases. In degenerative types, it decreases. It was not possible to correlate the histologic picture with the oxygen uptake.

152. The Design and Analysis of Clinical Studies of Caries-Inhibitory Agents. *Neal W. Chilton and John W. Fertig, School of Public Health of the Faculty of Medicine, Columbia University, New York, N. Y.* A frequently employed clinical study of caries-inhibitory agents utilizes one or more groups as experimental and one as control. A less frequently employed design, also important, utilizes one group of patients, with each individual acting as both treated and control. The data should be compiled at the end of the study period in a correlation table where the individuals are classified according to the original number of caries-free teeth and the number of teeth attacked during the study. Analyses then can be performed to determine whether the difference between treated and control groups is statistically significant by comparing the proportions of individuals whose teeth became carious, the average number of teeth becoming carious, and attack rates, or by means of analysis of covariance. The use of tooth surfaces as the unit of study is not recommended because of additional difficulties arising from the great and unassessed variation in caries attack for different surfaces. Pooling of teeth for comparison of over-all attack rates does not allow for proper assessment of variation for significance testing.

153. The Selective Action of Sodium Azide in the Cultivation of the Oral Species of Lactobacillus. *A. J. Clement, Dental School and Oral Hospital, University of Witwatersrand, Union of South Africa.* Standard tomato agar (pH 5.0) with which is incorporated sodium azide (1:10,000) has been under trial by several authorities as a selective medium for the cultivation of the lactobacillus genus. Saliva samples were collected from a group of European dental students by a standard technic. Colonies and morphologic characteristics of organisms from twenty-five inoculations of the azide-tomato agar medium were compared with twenty-five duplicate inoculations of the standard tomato agar (containing no azide). Both were incubated at 37° C. for four days. None of the azide plates were overgrown with yeasts or aerial contaminants, although two or three distinctive colony forms were observed on the majority of the azide plates. Microscopic identification showed that with very few exceptions (comprising streptococcoid and indefinite coccoid forms) these morphologically different colonies were composed of gram-positive rods varying in size from short cocci-bacilli forms to lengthy filaments. Filaments frequently grew alongside cocci-bacilli. It is suggested that the normal lactobacillus flora of the human mouth is comprised of species additional to the *Lactobacillus acidophilus*, a fact which may have been overlooked in earlier cultivation technics due to the presence of so many extraneous organisms. Twelve duplicate inoculations of Jay's glucose broth (pH 5.0) and the identical broth containing 1:10,000 sodium azide were also made. Determinations of pH were carried out with a glass electrode-calomel reference cell connected to a Cambridge pH meter reading to two

decimal places. After ten days' incubation at 37° C., the average value obtained for the ordinary broth inoculation was pH = 4.06 (range = pH 4.41 – pH 3.83) and for the azide broth pH = 4.04 (range = pH 4.48 – pH 3.81).

154. Field Studies in the Southern Kalahari: August, 1951. *A. J. Clement, Dental School and Oral Hospital, University of Witwatersrand, Union of South Africa.* Clinical findings on a group of seventy Bakalakadi (Bechuana) school children and various Kalahari bushmen subsisting on a primitive diet showed a low caries incidence (10 to 16 per cent) during childhod, adolescence, and early adult life. There is an increased susceptibility with advancing age, however. Presumably this feature is related to occlusal attrition and the disruption of the proximal contact points, which inevitably must increase the tendency toward food stagnation. A survey of the caries pattern showed the almost complete absence of caries in the anterior teeth. Marked endemic fluorosis was noted in several Kalahari settlements and checked against water analyses. The gingival and periodontal picture exhibited by the subjects demonstrated a greater susceptibility to various gingival and periodontal disturbances than is apparent in European subjects of a similar age group. Tartar formation was copious in many cases and very prevalent among the bushmen. It is felt that there may be a correlation between this feature and the highly mineralized pan-water normally consumed by these peoples. Tooth alignment was good. Chronologically the eruption of the teeth took place at an earlier age than in Europeans. Various biochemical tests were carried out on test samples of saliva. The most interesting finding was a higher average buffer capacity than is usually obtained with European samples. A comprehensive bacteriologic investigation showed an oral flora similar to that pertaining in civilized Europeans. One rather striking bacteriologic finding was a high percentage of agar plates containing aciduric gram-negative bacilli (provisionally identified as members of the *Bacterium coli-aerogenes* or *Pseudomonas pyocyanea* groups). The unhygienic environment in which these natives dwell is sufficient explanation for their frequent presence in the primitive oral flora.

155. Anthrone Carbohydrate Method for Determination of Food Retention. *Margaret R. Dewar, Hyman J. V. Goldberg, and Thomas G. Ludwig, Eastman Dental Dispensary, Rochester, N. Y.* The Somogoyi-Schaffer-Hartmann method previously used to determine the amount of carohydrates present in foods has the disadvantage of requiring hydrolysis of food prior to estimation of being influenced by reducing substances other than glucose and modified by the concentration of the test sample. The anthrone technic described by Morris and Morse was tested to determine accuracy and sensitivity and the effects of different sugars and proteins. Tests were made on samples of food and sugar and saliva mixtures. Satisfactory results indicate that the anthrone technic has advantages in permitting more speedy determinations, not requiring hydrolysis, and not being unduly affected by noncarbohydrate-reducing substances.

156. Microgasometric Estimation of the Salivary Gases. *H. Eggers-Lura, St. Elisabeth's Hospital, Holbaek, Denmark.* The salivary content of oxygen, nitrogen, and carbon dioxide has been examined by means of the Roughton-Scholander microgasometric method upon 100 persons under standardized conditions. The mean values for salivary oxygen content amounted to 1.35 vol. per cent ±0.0002 for caries-free and caries-resistant persons and 0.51 vol. per cent ±0.0003 for caries-active persons, the differences being highly significant. Seasonal variations corresponding to variations of the redox potential could be observed. The N_2 content of saliva varied from 0.48 to 2.78 vol. per

cent with slight relation to caries. The CO_2 values ranged from 13 vol. per cent to 85 vol. per cent, depending chiefly upon the rate of stimulation. The bicarbonate content of stimulated saliva accounted for far more than one-half of the total CO_2 content at pH 6.9.

157. A Study of the Effect of a Typical Infant's Diet on the Caries Incidence of the Syrian Hamster. *Howard C. Elliott, Jr., and Ward Pigman, University of Alabama, Dental School and Medical College, Birmingham, Alabama.* Many common infant formulas have compositions generally similar to those which have been found by numerous investigations to cause extensive development of dental caries in rats and hamsters. The usual infant formulas consist primarily of a type of commercially processed carbohydrate and of milk in some form with various types of vitamin supplements. In general agreement with earlier work, it is shown that diets composed mostly of whole dry milk (30 per cent) and sucrose and/or uncooked starch in varying proportions (66 per cent) cause the development of caries in hamsters. These results were obtained in a shorter period than usual by placing 17-day-old hamsters on the experimental diets for fifty-eight days. Every experiment was carried out at three different times, and each diet involved the use of about twenty-five animals, fairly evenly distributed according to sex. One common type of infant formula based on enzyme-hydrolyzed starch as the carbohydrate was tested for its cariogenic effect on hamsters. Although the total soluble carbohydrate was similar in amount to that of the cariogenic diets, relatively little caries was produced in fifty-eight days. This may be the result of the specific type of carbohydrate included in this formula or possibly it may be the result of the better nutritional qualities of this diet.

158. Glycogen Determination in Gingival Tissue. *B. K. Forscher, A. G. Paulsen, and W. C. Hess, Georgetown University School of Dentistry, Washington, D. C.* The glycogen content of gingival tissue from patients exhibiting periodontal pathosis was determined on 20-mg. tissue samples by a combination of the Johnson wet ash technic to determine sample weight and the anthrone procedure of Seifter and associates to determine glycogen. The usefulness of the Johnson wet ash method for analysis of small samples should be emphasized for dental research. Glycogen values in pathologic specimens exhibited a rather wide variation: from 0.1 per cent to 0.4 per cent with occasional higher values, and with a mean value of 0.283 per cent. The few cases of acute periodontal involvement appeared to run higher in glycogen than the main group of cases which were classified as chronic on the basis of clinical observation. Each sample was composed of tissue adjacent to a periodontal pocket, the position and depth of which were noted prior to biopsy. When the glycogen content of the samples was arranged according to the depth of the pocket, the glycogen content was found to vary inversely with pocket depth. There was no apparent relationship between the glycogen content and position.

159. Oral Disorders Resulting From Electroshock Therapy With a Device for Better Oral Protection. Preliminary Report. *J. J. Freedland and W. J. Carter, USN Dental Research Facility, Great Lakes, Ill.* Guards and gags currently used for protection of the hard and soft tissues of the mouth during electroshock therapy were investigated. Most of these appliances were found inadequate, while others contributed to the hazards of oral injury. In 121 cases observed forty-one individuals exhibited mild to severe gingival hemorrhage, forty-five individuals experienced some degree of cyanosis, four individuals terminated treatment with mandibular subluxations, and five showed tongue

and lip lacerations. Patients without any form of guard were tested by means of a gnathometer, resulting in a reading approximately three times their normal closure force. A universal guard has been designed and fabricated for use in this type of therapy and is suggested for other fields of medicine or dentistry requiring oral protective devices.

160. A Histochemical Study of the Effects of Cortisone on the 20-Day-Old Fetal Rat Incisor. *E. D. Goldsmith, New York University College of Dentistry and Office of Naval Research, New York, N. Y., and Leonard Ross, New York University, New York, N. Y.* On the seventh day of gestation twelve rats were divided into three groups of four each. The animals in Group I received 15 mg. cortisone (Cortone, Merck) subcutaneously each day; those in Group II each received 20 mg. cortisone, and Group III served as controls. By means of cesarean section 14-, 16-, 18-, and 20-day-old fetuses were removed. Of the total number of 115 fetuses, twenty were 20 days old. Of these, six were in Group I, seven in Group II, and seven in Group III. The fetuses were fixed in cold 95 per cent alcohol, processed by the cedarwood oil-paraffin technic and the mandibles were sectioned at 7 μ. The Gomori alkaline phosphatase, the methyl-green pyronin DNA and RNA, and the McManus periodic acid glycogen methods were used for histochemical visualization. The lower incisors of the 20-day-old cortisone-treated fetuses exhibited an accelerated differentiation and organization of the ameloblastic and odontoblastic layers. There were also signs of increased dentin deposition. The alkaline phosphatase activity in the stellate reticulum underlying the labial ameloblasts showed an increase, as did the glycogen in this region. The typically high RNA content of the odontoblastic and ameloblastic layers in the labial side of the tooth was further increased in the cortisone-treated animals. Further studies are in progress, utilizing earlier and later stages in tooth development.

161. Effects of Long-Term Cortisone Treatment on the Supporting Dental Structures of the Rat. *E. D. Goldsmith, New York University College of Dentistry and Office of Naval Research, New York, N. Y., and S. S. Stahl, New York University College of Dentistry, New York, N. Y.* Twenty-four rats, eight males and sixteen females weighing an average of 130 Gm. at the beginning of the experiment, were divided into four groups and treated as follows: Group I, four males and four females were given 0.5 mg. of cortisone acetate (Cortone, Merck) subcutaneously daily; Group II, four males and four females were given 3.0 mg. of cortisone subcutaneously daily (the males in this group succumbed prior to the termination of this experiment); Group III, four males and four females were given 0.6 c.c. of 0.9 per cent NaCl subcutaneously daily; Group IV, four females were maintained as uninjected controls. At the end of one year, all animals were sacrificed. The mandibles were excised and fixed in Bouin's fluid and decalcified by the formic acid-sodium citrate technic, embedded in paraffin, and sectioned at 7 μ. All sections were stained with hematoxylin and eosin. In the Group III animals which received 3.0 mg. of cortisone daily, histologic study revealed an increase in the amount of alveolar bone and a marked narrowing of the marrow spaces. This appearance may be the result of a retardation of endosteal resorption. In the periodontal membrane a disorganization of the arrangement of the fibers and their attachment to the bone was observed. No changes were seen in the animals of Group I treated with 0.5 mg. of cortisone daily. (The animals were treated and generously supplied by Dr. C. A. Winter of the Merck Institute for Therapeutic Research.)

162. Nucleic Acid Content of Human Gingiva. *Wesley Halpert, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* As part of

a study on the etiology of periodontal disease, desoxyribonucleic acid was determined in human gingiva. The Feulgen reaction, in which desoxyribonucleic acid regenerates the color of basic fuchsin which has been decolorized by sodium metabisulfite, was used to determine the desoxyribonucleic acid in the individual nuclei of histologic sections. The absorption of light by the stained nuclei can be measured and is proportional to the amount of desoxyribonucleic acid present. The apparatus employed consists of a monochromatic light source, a microscope and a photoelectric cell. Results are calculated as follows: $T = \frac{I}{I_0}$, where T = transmission, I = light passing through a plug of tissue, I_0 = light passing through a clear area next to the tissue plug. $E = \log \frac{1}{T}$, where E = extinction. E is proportional to the number of absorbing molecules. To eliminate as many errors as possible, four pieces of tissue, namely, one normal, one from a case of periodontosis, one from a case of periodontitis, and one rat liver, were embedded in each paraffin block. Therefore, comparisons of the relative amounts of desoxyribonucleic acid in these tissues were made on the same slide. Nuclei from the basal layer of the epithelium of fifteen specimens have thus far shown no difference between the normal, periodontosis, and periodontitis specimens.

163. Variability of Chemical and Biologic Properties of Various Water-Soluble Chlorophyll Derivatives *John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* In an attempt to discover the mechanism of the bacteriostatic action of water-soluble derivatives of chlorophyll, certain chemical and physical properties of over thirty different samples of copper chlorophyllin collected from 1947 to 1952 were compared with the effect on salivary acid production from carbohydrate in vitro. It was found that in all samples but one the copper content was less than the theoretical amount and in most cases was only slightly over 50 per cent of the theoretical amount. While the estimated purity, base content, water content, copper content, and absorption spectra characteristics of the samples varied widely, not one of these properties was related to in vitro activity. It was found that when water solutions of the chlorophyllins were precipitated by lowering the pH to 3 with HCl, certain samples released varying amounts of copper. The chlorophyllins which released the most copper were in general found to be the most effective in vitro. However, the redissolved chlorophyllins and those which did not release copper possessed definite inhibitory action in vitro. Thus, certain samples of the copper water-soluble derivatives of chlorophyll may effect bacterial activity in at least two ways. It is evident that investigators studying these compounds should endeavor to rule out the effects of any free or loosely bound copper component.

164. Lactobacilli and Dental Caries in the Hamster. *Edith Zak Helman and David F. Mitchell, University of Minnesota, Minneapolis, Minn.* Since lactobacillus counts are indicative of current and possibly future caries activity in human beings, we wished to see if this is also true in hamsters. If such a relationship was found in hamsters, a useful tool would be available by which it would be possible to determine the influence of various factors on caries incidence before the end of the usual experimental period. A micro method for the determination of lactobacilli and other organisms in hamster saliva was developed, as well as a simplified method for scoring caries, in which a grid is used in the eyepiece of the microscope to measure the area of the carious lesions. No correlation was found between caries experience and oral lactobacillus counts of forty adult hamsters maintained on three different diets (nonsugar, high-sugar,

and nonsugar plus water containing 20 ppm of NaF) for 100 days. A limited number of offspring of these females experienced the influence of the diets just mentioned until 21 days of age and were then fed a high-sugar diet for 100 days. Significant correlation ($P = 0.007$) was obtained between the occlusal scores and lactobacillus counts taken one month prior to sacrifice, but not between the occlusal scores and La counts taken at the time of sacrifice. It would be tempting to postulate an early caries association with La in the hamster. However, contamination by fecal La as a result of coprophagy leaves the significance of the findings open to question. This is substantiated by the fact that a significant correlation ($P = 0.0001$) was found between the lactobacillus and coliform counts in the saliva in the adult hamsters.

165. A Preliminary Survey of Periodontal Disease Among Basic Airmen. *Marvin Jacobson, School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.* A PMA survey was made on 670 incoming basic airmen at Lackland Air Force Base during medical processing. The average age of the men was 19.1 years. Approximately 85 per cent of the airmen were found to have gingivitis to some degree. The PMA survey was calculated on a percentage basis, taking into consideration the number of teeth found in the mouth: 32 teeth, 30 papillary units, 32 marginal units, and 32 attached gingival units constituted a complete dentition (100 per cent). Among those having gingivitis, 35 per cent of the papillary gingivae, 14 per cent of the marginal gingivae, 1 per cent of the attached gingivae, and 16 per cent of the combined PMA gingivae in any one mouth were affected. Papillary gingivitis was found most frequently in this survey. The area involved most often with papillary gingivitis was the upper right molar region. The lower incisor-cuspid region was next in order of severity. Marginal gingivitis was found most frequently in the upper and lower anterior regions. Attached gingivitis was seldom found in this survey. Because of the great number of men afflicted with some degree of periodontal disease, it is advocated that these airmen receive immediate and thorough prophylaxis, education and instruction in the proper method of oral hygiene, and necessary periodontal treatments to prevent any further breakdown of the dental supporting structures. All of these measures will aid in eliminating acute conditions in the infected oral tissues of the airman, which otherwise would hamper the efficiency of his performance during the weeks of basic training and thus adversely affect his entire service career.

166. A Study of Gingival Exfoliative Cytology and Its Possible Use in the Diagnosis of Periodontal Disease. *Marvin Jacobson, School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.* Thirty-five gingival smears and biopsies were obtained and classified: Normal, papillary inflammation, papillary-marginal inflammation, papillary-marginal-attached inflammation, acute necrotizing, chronic necrotizing, and hyperplastic. Separate smears of the attached, marginal, and pocket gingivae were made, then fixed and stained according to the Papanicolaou technic. Biopsies were taken of this gingival tissue and fixed in Carnoy's fluid or frozen with liquid nitrogen and subsequently dehydrated. Sections were stained with Papanicolaou, hematoxylin and eosin, and periodic acid Schiff-Macmanus stains. Exfoliated cells studied were put into the following classes: (1) Totally keratinized (no nuclei, and stained either green-blue, yellow-brown, or red-orange); (2) outer superficial (pyknotic nuclei, and stained either green-blue, yellow-brown, or red-orange); (3) inner superficial (larger nuclei having membrane with visable structures and a large amount of cytoplasm staining green-blue, yellow-brown, or red-orange); (4) intermediate (large nuclei, smaller amount of cytoplasm, and stained green-blue); (5) outer basal (small amount of cytoplasm with large

round or oval nucleus staining green-blue); (6) basal (small cells with large, round nucleus and small amount of cytoplasm staining green-blue); (7) inflammatory elements (tissue and blood varieties). Smears of attached, marginal, and pocket gingivae showed completely different pictures, more obvious in diseased states. The normal smear contained mostly Class 1 cells in attached gingiva, Class 2 and 3 cells in marginal gingiva, and Class 2 and 3, and a few Class 4 cells in pocket gingiva, along with various bacterial forms, degenerating histiocytes, and epithelial cells. Inflamed and necrotic smears contained Class 2 and 3 cells in attached gingiva, Class 2, 3 and 4 cells in marginal smear, along with varied numbers of bacteria. Pocket gingiva had Classes 3, 4, 5, and even 6, along with various histiocytes and many more bacterial forms of all varieties. These smears were all correlated with biopsy section, and the origin of the cells seen in the smear became quite obvious, as well as the particular staining characteristics of the cells in the smear.

167. A Preliminary Histologic and Cytologic Survey of Normal and Pathologic Human Gingival Tissue by the Use of Various Fixatives and Stains. *Marvin Jacobson, School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.* Thirty-two gingival biopsies were studied by the use of five different fixatives and five different stains. In all, 160 sections were analyzed. Fixatives used were 10 per cent neutral formalin, 20 per cent neutral formalin, Bouin's fluid—Allen's PFA-3 modification, Zenker's acetic fluid, and absolute alcohol. The stains used were hematoxylin and eosin, Van Gieson, Heidenhain's iron hematoxylin, periodic acid Schiff-Macmanus, and the Brown-Brenn bacterial stain. The gingival biopsies were classified into the following groups: apparently normal, mildly inflamed, hyperplastic, acute necrotizing ulcerative, chronic necrotizing ulcerative, and clinically uninvolved within a diseased mouth. Most interesting findings were seen in clinically uninvolved biopsies. Definite cytologic and histologic changes occurred within the gingiva of this group, even though no apparent clinical signs were as yet evident. Inflammatory changes of the gingiva from an acute to a more chronic necrotizing ulcerative state were also observed. Generally Zenker's acetic fixation was found best for studying epithelium and also for staining tissues by the Brown-Brenn procedure. Bouin's fixation was best for nuclear detail of epithelial cells and inflammatory elements. Absolute alcohol was best when studying connective tissue as well as glycogen content and basement membrane. Definite combinations of fixative and stain should be considered when a particular cytologic structure is being investigated in both normal and diseased states.

168. Determination of Oral pH in the Syrian Hamster. *Erling Johansen, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y., and Morrison Rogosa, National Institute of Dental Research, Bethesda, Md.* The purpose of the study was to determine (a) the pH of the secretions of the major salivary glands and (b) the pH of certain areas of the molar teeth and surrounding soft tissues. A microantimony electrode made according to the specifications of Thompson and Brudevold was employed. The animals were immobilized so that the specific area under study was observed by means of a wide-field microscope at a magnification of 12×. The following values from 120 different animals on stock diet (Purina Rabbit Pellets) and caries-producing diet (CPD), in which no significant differences were found between the two groups, represent averages and ranges of twenty to fifty readings in each specific area: parotid duct opening 9.0 (8.3 to 9.9); sublingual duct opening 8.6 (8.1 to 9.4); palate 8.5 (7.9 to 9.0); posterior to third molar 8.1 (6.9 to 9.0); occlusal surfaces of sound teeth in animals free of caries 8.5 (7.9 to 9.2). The following readings are from animals on the CPD only: periodontal pockets 8.5 (7.5 to

9.1); carious lesions with saliva in situ 7.5 (5.3 to 9.0): sound surfaces on carious teeth with excess saliva removed 8.0 (6.8 to 8.7); and carious lesions on same teeth with excess saliva removed 6.5 (5.1 to 7.8). Check readings of the sublingual and parotid duct areas with a glass electrode gave readings slightly lower than the antimony electrode. Sublingual and palate readings were within 0.2, whereas the parotid results were within 0.5 pH unit. Readings on standard buffers were in good agreement with both electrodes. Appropriate indicators introduced into the oral cavity gave color changes in the areas studied, confirming the general range of pH values.

169. Effect of Strontium Chloride on Experimental Dental Caries in the Syrian Hamster. *Erling Johansen and John W. Hein, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.* An experiment has been conducted to determine the effect of strontium on experimental dental caries in the Syrian hamster. Forty-two male and forty-two female hamsters 35 days old were placed by litter-mate distribution into two groups and maintained on a caries-producing regimen for 175 days. Group I received Sr as strontium chloride in amounts of 50 ppm in the drinking water. Group II received distilled water. At the end of the experimental period, the animals were examined for dental caries in vivo and scored according to a modification of Keyes' system. The resulting scores with standard deviation were: Group I, twenty-one males, Sr 50 ppm, 46 ± 44; Group I, twenty-one females, Sr 50 ppm, 37 ± 51. Group II, twenty-one males, no Sr, 49 ± 39; Group II, twenty-one females, no Sr, 33 ± 58. From these data it is concluded that Sr in amounts of 50 ppm administered in the drinking water is ineffective in reducing experimental dental caries in the Syrian hamster.

170. Effect of Detergents Added to Fluoride Solutions Used for Topical Applications. *Maury Massler, University of Illinois, Chicago,* Freshly extracted teeth were used to test the effect of detergents added to fluoride solutions when these were used for topical applications on intact whole enamel according to the experiment design just outlined. It was found that the addition of a variety of cationic and anionic detergents including sodium lauryl sulfate and Zephiran to any one of the fluoride solutions tested decreased the time required to etch the enamel. These detergents thus interfered with and decreased the ability of the fluoride solution to protect the enamel against acid action. For example, immersion of intact enamel in sodium silicofluoride (saturated) for one hour protected the enamel against etching by lactic acid pH 3.9 for 240 minutes. Addition of sodium lauryl sulfate to the silicofluoride solution decreased the time required to etch the enamel to 72 minutes. It is therefore concluded that detergents of this type inhibit or interfere with the action of fluoride salts when applied to the enamel in vitro.

171. Effect of Various Fluoride Salts in Inhibiting Acid Action on Intact Enamel. *Maury Massler, University of Illinois, Chicago, Ill.* Freshly extracted teeth were used to test the relative efficiency of various fluoride salts in inhibiting the etching action of acid on intact enamel surfaces. One-half of the cleaned enamel surface was immersed in the fluoride solution for various periods of time, while the other half served as the nonfluoridized control. The whole tooth was then placed in lactic acid buffered to pH 3.9, and the time required to etch the enamel was recorded. The nonfluoridized enamel of adult permanent teeth etched in an average of 15 (±3) minutes. Intact adult enamel placed in sodium fluoride 2 per cent solution for 24 hours resisted etching of the surface for 90 (±10) minutes. Forty fluoride salts were tested in this manner.

A number of fluoride compounds were found to be more effective than sodium fluoride. Sodium silicofluoride was the most effective of the salts tested in protecting the adult enamel against lactic acid action (etching time was 60 [±10] minutes after 5 minutes' immersion, 240 [±10] after 1 hour's immersion, and 3,000 minutes after 24 hours' immersion). Clinical studies are now in progress to test the in vivo effectiveness of topically applied sodium silicofluoride solution in protecting the enamel against dental caries.

172. Relation of Gingival Inflammation to Alveolar Crest Resorption. *Maury Massler, H. R. Muhlemann, and Isaac Schour, University of Illinois, College of Dentistry, Chicago, Ill.* The degree of inflammation of each gingival papilla and the radiographic appearance of the corresponding alveolar crest were analyzed and compared in 350 young adult males. The gingival papilla was rated as showing no inflammation, mild, moderate, or severe inflammation. The corresponding alveolar crest was rated from roentgenograms as showing no resorption, slight resorption (crest blunted, with no lamina dura, within the cervical quarter of the root), moderate resorption (crest within second quarter of root), severe resorption (crest below midroot level, within third quarter of root) or very severe resorption (crest at apical quarter of root). No relationship could be established between the degree of inflammation of the gingival papilla and the level or the radiographic appearance of the underlying alveolar crest. When a group of 100 patients with no gingivitis was compared with a group of 100 patients with moderate to severe gingivitis, it was found that there was a slightly greater number of alveolar crests showing resorptions in the group with gingivitis. However, no prediction as to the condition of the alveolar crest could be made from the clinical examination of the gingivae, nor could the condition of the gingivae be predicted from the roentgenographic examination of the alveolar crest.

173. A Study of Various Methods of Collecting Hamster Saliva for Bacterial Counts. *Herman Medak and George W. Burnett, Department of Dental Research, Army Medical Service Graduate School, Washington, D. C.* A comparison has been made of various methods of collecting hamster saliva on lactobacilli counts. The methods of collection were (1) by swabbing the oral cavity for one minute, then shaking the swab mechanically for thirty minutes in broth; (2) by swabbing the oral cavity for one minute, then thoroughly squeezing the swab against the wall of the tube of broth; (3) by obtaining saliva from the oral cavity with a medicine dropper whose opening was 1 mm. in diameter until no more could be collected; and (4) by collecting pilocarpine-stimulated saliva by Method 3. Weighed saliva samples cultured on Rogosa's medium were collected by each of the four methods from four separate groups of five adult caries-free hamsters on five consecutive days. In turn, each experiment was repeated three times with additional groups of animals. The greatest amount of saliva was collected by Method 1 and in decreasing amounts by Methods 3, 4, and 2. The locatobacilli counts varied as much as 100 per cent in each animal from day to day, but with less variation between each method of collection. When the numbers of lactobacilli were calculated quantitatively on the basis of the amount of saliva collected, more were obtained by Method 1, and fewer with Methods 3, 4, and 2. When the lactobacilli were calculated qualitatively on the basis of the total unweighed sample, more lactobacilli were obtained by Method 1 and fewer with Methods 4, 3, and 2, but with much variation between each method of collection. It is suggested from these data that saliva samples must be obtained by reproducible and quantitative methods if valid lactobacilli counts are to be obtained.

174. Studies on the Location of the Lingula, Mandibular Foramen, and Mental Foramen. *James A. Miller, Jr., Emory University School of Dentistry, Atlanta, Ga.* Mandibles of seventy-five Hindu skulls (estimated ages 20 to 45 years) with full or nearly full dentition were studied. The average position of the lingula is 34 per cent of the distance from mandibular notch to notch in front of gonium (range: 24 per cent to 47 per cent). Its anteroposterior location is behind middle of ramus (range: 43 per cent to 76 per cent of distance from anterior border). The mandibular foramen is on the average almost exactly midway between mandibular and gonial notches (range: 31 per cent to 58 per cent of the distance). It is posterior to the middle of the ramus (range: 45 per cent to 71 per cent of distance from anterior border). Variability in ramus width (range: 24 mm. to 39 mm.) and in distance from anterior border to mandibular foramen (range: 12 mm. to 25 mm.) dictates that no absolute figure for depth of insertion will be satisfactory. The mental foramina were located: below apex of first premolar, 3 per cent; between first and second premolars, 38 per cent; below apex of second premolar, 40 per cent; between second premolar and first molar 20 per cent. The relationships of these findings to mandibular block and mental block anesthesia will be discussed.

175. Effects of Urea on the Hamster. *David F. Mitchell and Edith Zak Helman, University of Minnesota, Minneapolis, Minn.* In a previous paper it was reported that 0.6 per cent urea in a caries-producing diet had no effect on dental caries or periodontal disease in the hamster. In this study the effects of higher concentrations of urea were studied in regard to weight, kidney size and microscopic structure, water intake, periodontal involvement, and caries incidence. A pilot study to determine the toxicity of urea was done. Caries-producing diets containing up to 20 per cent urea did not affect the weights of young or adult hamsters maintained on such diets for 100 days, as compared to controls. Diets containing 50 to 75 per cent urea proved fatal to young hamsters within a week. Water consumption paralleled the percentage of urea ingested, and such a parallel also was evident in the ratio of kidney size to body weight. The extent of kidney hypertrophy, however, even in the 20 per cent urea animals, was slight, and no microscopic changes in the kidneys were noted in any of the animals ingesting up to 75 per cent urea. One hundred twenty 30- to 50-day-old hamsters were then separated according to sex and divided into three groups: (A) control (caries-producing diet); (B) caries-producing diet containing three per cent urea; and (C) caries-producing diet containing 9 per cent urea. No difference in either periodontal involvement or caries scores was evident between the three groups; however, since the caries scores of the control and experimental animals were of a low order, further studies are under way to determine the efficacy of urea as a caries-reducing agent.

176. Fracture Lines in the Mandible. A Comparison of Roentgenologic and Gross Anatomic Findings. *Eli Olech, College of Dentistry, University of Illinois, Chicago, Ill.* The purpose of this study was to correlate fracture lines as they appear in roentgenograms of fractures of the mandible and as they exist in gross anatomic specimens. Six wet specimens of human mandibles were used. A single fracture was produced by means of a disk. In two specimens the plane of the fracture was transverse through the body of the mandible at a right angle to the outer surface of the body of the mandible, in two the fracture plane was oblique, and in two the fracture was through the angle of the mandible, one of the fracture planes being at a right angle and one at an oblique angle to the outer surface of the mandibular body. The conclusions reached were: (1) In roentgenograms of a single fracture of the

mandible with no displacement of the fragments, unless the x-rays are parallel to the plane of the fracture, two fracture lines which usually meet at their superior and inferior borders may be seen. (2) In an oblique fracture of the mandible, two fracture lines are always visible in the roentgenogram unless the plane of the x-rays is parallel to the plane of the fracture. (3) In determining the number of fractures present in a fractured mandible, a minimum of three extraoral roentgenograms should be taken from (1) a routine position, (2) an anterior position, and (3) a posterior position. In addition, an anteroposterior film (for condylar fractures) and intraoral views, both routine and occlusal, may be helpful. Extreme caution must be used in attempting to diagnose the number of fractures present in a fractured mandible from the number of fracture lines appearing in the roentgenogram.

177. The Amino Acid Content of Dentin Protein. *K. A. Piez, National Institute of Dental Research, Bethesda, Md.* As a preliminary to studies on possible variations in dentin and enamel proteins under different conditions of formation and maintenance, a complete amino acid analysis of dentin protein was carried out. Crowns from sound human teeth were ground and the dentin was separated by flotation. The protein was obtained in good yield by decalcification with a neutral solution of ethylenediaminetetraacetic acid. After acid hydrolysis, the amino acids were separated by ion exchange chromatography and individually quantitated by the ninhydrin method. About half of the protein consisted of glycine, proline, and hydroxyproline. Hydroxylysine was present. The results are in good agreement with other analyses of dentin protein and of collagen from other sources.

178. Evaluation of Antifungal Agents Used in Endodontics. *O. Pita, Louis I. Grossman, and C. McShane, University of Pennsylvania Dental School, Philadelphia, Pa.* Six antifungal agents, three antibiotics, and three antihistamines were evaluated for relative effectiveness by the serial dilution method. Of these fradicin was found most effective against *C. albicans*.

179. I. A Comparison of the Volume of the Left and Right Maxillary Sinuses in the Dog. *Marvin Rosen and Bernard G. Sarnat, University of Illinois, College of Dentistry, Chicago, Ill.* The purpose of this investigation was to compare the volumes of the left and right maxillary sinuses in the dog. Ten normal adult dogs with complete dentitions were sacrificed and their heads were immediately severed, fixed, and sectioned in the midsagittal plane. A low-fusing-point metal was poured into the maxillary sinus of each half via its ostium. The casts were then delivered by removal of the medial wall of the sinus and the excess trimmed at the level of the ostium. From the casts the volumes of the sinuses were determined. It was found that in five of the dogs the volumes of the left and right maxillary sinuses differed less than 3 per cent. In the other five the differences ranged from 7 to 16 per cent. In three of these the left sinus was larger (8.4, 13, and 16 per cent) than the right. In the other two the right maxillary sinus was the larger (7, and 9.7 per cent).

180. II. Change of Volume After Extraction of Teeth Adjacent to the Maxillary Sinus in the Dog. *Marvin Rosen and Bernard G. Sarnat, University of Illinois, College of Dentistry, Chicago, Ill.* The purpose of this investigation was to compare the volumes of the left and right maxillary sinuses in the dog after extraction of the teeth adjacent to the left maxillary sinus. In a previous report it was demonstrated that the volumes of the left and right maxillary sinuses in the normal dog were essentially the same. In ten normal adult dogs with complete dentitions, the teeth (upper left third and fourth pre-

molars and first and second molars) immediately adjacent to the maxillary sinus were extracted. The dogs were sacrificed 6 to 12 months later and their heads immediately severed, fixed, and sectioned in the mid-sagittal plane. A low-fusing-point metal was poured into the maxillary sinus of each half via its ostium. The casts were then delivered by removal of the medial wall of the sinus and the excess was trimmed at the level of the ostium. From the casts the volumes of the sinuses were determined. It was found that in two of the experimental animals the volumes of the left and right maxillary sinuses differed by less than 2 per cent. In the other eight dogs the differences ranged from 4.5 to 27 per cent. In seven of these eight animals the maxillary sinus was significantly larger on the side from which the teeth were extracted (6.7, 7.1, 9, 10.5, 19, 20, and 27 per cent). In only one animal was the maxillary sinus larger on the unoperated side (4.5 per cent).

181. The Application of Varidase* in Dentistry. *Lester Hugh Roth, School of Dentistry, University of Pittsburgh, Pittsburgh, Pa.* Patients who had demonstrated a reduction of suppuration following antibiotic treatment of acute dental alveolar conditions showed further improvement with the use of Varidase. Treatment of aphthous stomatitis cankers with Varidase resulted in improvement, but not conclusive evidence of shortening the duration of lesions. Oral fistulas resulting from acute alveolar abscesses responded to Varidase treatment. Treatment of idiopathic lesions of the oral mucosa resulted in varying degrees of improvement. Pyrogenic reactions occurred in the early investigations, but methods of allaying and reducing these reactions have been determined.

182. A Fourteen-Year Report of Facial Growth in a Case of Complete Anodontia With Ectodermal Dysplasia. *Bernard G. Sarnat, Allan G. Brodie, and W. Howard Kubacki, University of Illinois, College of Dentistry, Chicago, Ill.* The importance of the deciduous and permanent teeth in the development of the face and jaws has been a controversial problem. A patient with complete anodontia and ectodermal dysplasia has been studied from 2 to 16 years of age. Five sets of full upper and lower dentures were designed, constructed, and delivered. Each successive denture was larger and contained more and larger teeth to accommodate the increase in the size of the jaws. Appraisal of serial cephalometric roentgenograms taken at 6- to 12-month intervals during childhood and adolescence revealed the patient's over-all measurements equal to at least small normal. A slightly less than normal nasion-anterior nasal spine dimension coupled with a markedly greater-than-normal vertical measurement within the nasal cavity proper gave the impression of a buckling of the palate. The nasal cavity and accessory sinuses appeared to be overexpanded, and the bones appeared to be fragile. Whether this is symptomatic of this type of dysplasia or is traceable to a general weakening of the facial skeleton induced by a lack of natural teeth is a question that must await further investigation.

183. Restorative Needs of Primary Teeth of Nursery Children 1 to 6 Years of Age. *Bhim Sen Savara and Theodore Suher, University of Oregon Dental School, Portland, Ore.* Dental examinations (def teeth) were made of 650 children in different nurseries in Portland, Ore. The examination revealed that 61 per cent of 3-year-old children had one or more decayed teeth. Whereas 4 per cent had a few restored teeth, not a single child had received complete dental attention. Total needs per 100 3-year-old children were 272 decayed teeth, of which nine were restored. At 4 years of age, 71.1 per cent of

*Lederle's name for Streptokinase-Streptodornase.

the children required dental attention, and of these only 4.2 per cent had completely restored mouths. At 6 years, 83.3 per cent of the children required fillings, of whom only 15.2 per cent had completely restored mouths, representing 159 restorations per 100 children. From 3 to 6 years of age, the incidence of dental caries increased from 273 to 513 def teeth per 100 children, while the number of restorations increased from 9 to 159 filled teeth per 100 children. The ratio of filled teeth to def teeth reduced from 1:33 at 3 years to 1:3 at 6 years, but the total number of unfilled teeth was 264 at 3 years and approximately 360 at 4, 5, and 6 years. The analysis of the data also included the percentage of children with dental caries and no restorations, as well as percentage of children with dental caries and partially restored mouths.

184. The Periodontium and Salivary Glands in the Alarm Reaction of Selye. *Gerald Shklar and Irving Glickman, Tufts College Dental School, Boston, Mass.* The alarm reaction, the initial phase of the general adaptation syndrome, was induced in a group of albino rats using subcutaneous injections of 4 per cent formaldehyde into the groin as the stressor agent. After 48 hours, the animals were sacrificed and the jaws, femur, adrenals, thymus, spleen, cervical lymph nodes, and salivary glands were prepared for microscopic study. The findings of enlargement of the adrenal cortex, involution of lymphatic tissue, and gastrointestinal ulceration were interpreted as indices of the existence of the alarm reaction. Salivary gland changes in the alarm reaction consisted of reduction of interacinar connective tissue and degeneration of acinar cells. There were no notable gross or microscopic changes in the periodontium.

185. The Effect of Manganese in the Diet on Dental Caries in the Rat. *Robert M. Stephan and M. Rachel Harris, National Institute of Dental Research, Bethesda, Md.* Experiments on white rats were made to determine the effect of varying the manganese concentration of the diet upon the development of carious lesions. Three synthetic diets were used, consisting of 20 per cent casein, 72 per cent dextrose, 3 per cent cottonseed oil, 0.2 per cent cystine, 0.2 per cent tryptophane, required vitamins, and 4 per cent of one of three salt mixtures. These salt mixtures were made up on the Osborne and Mendel formula, but in one the manganese was omitted, in another it was kept at the usual level, and in a third it was increased so that the final diet would contain 0.1 per cent manganese. Seven pregnant rats were placed on each of the diets, and the offspring were continued on the same diets for approximately 100 days. Result: Of thirty-three rats on the diet without manganese, twelve developed caries, with forty-two carious teeth in all. Of thirty-nine rats on the usual level of manganese (0.0003 per cent of diet) eleven developed caries, with thirty-three carious teeth in all. Of thirty-eight rats on the diet with 0.1 per cent manganese, five developed caries, with nine carious teeth in all. Thus, the addition of manganese appeared to decrease the development of caries, rather than to increase it. The average growth rates for the three groups were 0.85, 0.99, and 1.31 Gm. per day, respectively. Incisor pigmentation scores were 1.6, 2.4, and 3.5, respectively, where 4.0 represents maximum pigmentation. Calculus formation on the teeth and periodontal destruction were comparable on the three diets.

186. The Effect of Subminimal Magnesium on the Syrian Hamster. *George M. Yamane and Leon Singer, School of Dentistry, University of Minnesota, Minneapolis, Minn.* Six pairs of hamsters, distributed according to sex and litter mates, were kept on varying levels of magnesium and distilled water. A synthetic diet, adequate in all respects except for mangnesium, was given each group. Group I received 12 ppm of magnesium; Group II, 15 ppm; Group III, 20 ppm; Group IV, 25 pm; Group V, 35 ppm; and Group VI (control),

665 ppm. Because of the small number of control and experimental animals, the results obtained are not conclusive. Many of the findings are in agreement with the investigations of Tufts and Greenberg. All experimental animals showed hyperirritability, hyperexcitability, vasodilatation, convulsive seizures, cessation of growth, kidney damage, and hepatitis, and eventually they died. Duration of deprivation, rather than amount of deficiency, determined the severity of the diseased condition. Histologically the liver showed hyperemia, lymphocytic infiltration in the portal triads, and atrophy of the liver cells. The lung showed hyperemia and thickening of the interspetal wall. The kidney, which showed the most marked changes, developed hyperemia with necrosis of the parenchyma, particularly in the medulla. In the femur, the parallel columns of the bony spicules, normally seen in the metaphysis of the bone, were lost. The zone of preliminary calcification just below the epiphyseal line was either atrophic or absent. Osteoblastic activity was at a minimum. The mandible contained an abundance of marrow spaces and the alveolar crests were resorbed. Alternate bands in the dentin were seen associated with an area of odontoblastic degeneration at the middle third of the pulp of the lower incisors.

187. The Concentration of Citrate in Whole and Parotid Human Saliva. *I. Zipkin, E. H. Fath, and Robert M. Stephan, National Institute of Dental Research, Bethesda, Md.* A fasting sample of paraffin-stimulated saliva (A) was collected before breakfast from twelve adult subjects. On another day, a nonfasting sample of paraffin-stimulated saliva (B) was collected between 9:00 A.M. and 10:00 A.M. Similarly, on still another day between 9:00 A.M. and 10:00 A.M., paraffin was chewed, but with modified Lashley cups in place over both ducts of Stenson, so that both the parotid (C) and the extraparotid (D) nonfasting salivas could be collected simultaneously. All salivas were collected in 0.5 ml. concentrated sulfuric acid, since previous work has shown that citrate in freshly collected whole saliva will decrease to 20 per cent of its original concentration within one hour. All saliva collections were repeated at least three times at approximately one- to two-week intervals. The mean values in milligrams per cent for the twelve subjects were 0.52, 1.05, 1.47, and 0.36 for salivas (A), (B), (C), and (D), respectively. In every case the paraffin-stimulated fasting salivary citrate level was lower than the nonfasting sample collected between 9:00 A.M. and 10:00 A.M. The parotid salivary citrate was the highest reached in every case. The lowest citrate concentration was invariably found in the extraparotid saliva so that the descending order of salivary citrate concentration was (C), (B), (A), and (D).

VI. Abstracts of Papers Read in Materials Group Sessions

188. Manufacture of Steel Dental Burs. *K. H. Strader, Ransom & Randolph Company.* The various steps involved in the manufacture of steel dental burs are described. The steel used, its dimensional specifications, effect of chemical analysis, and machinability characteristics are covered. Numerous types of equipment are involved in machining operations. Automatic screw machines of various types are used for the blanking operation. Teeth or flutes are formed by various types of "head cut" milling machines for the different bur designs. A number of single-purpose machines are required. Hardening is usually done by salt bath treating. Final stages of production include polishing, inspection, and packaging. Some general remarks on design are included.

189. A Study of the Relationship Between the Design and Cutting Efficiency of Dental Burs. *E. E. Henry and F. A. Peyton, University of Michigan, Ann Arbor, Mich.* This is the report of a preliminary study between

the design and cutting efficiency of a dental bur. This study deals with the cutting efficiency of dental burs (No. 557 fissure and No. 37 inverted cone burs) as determined by tests using Ivoryene and brass as the material being cut. Ivoryene is a soft material which allows the cutting efficiency to be studied without appreciably dulling the dental bur. Studies in brass show a dulling effect. The relationships between the cutting efficiency of the bur and the depth of cut and variations of speeds of rotation are explored. The life of the tungsten carbide dental bur and the life of a steel bur are also compared. Photographs of the various products are made with both longitudinal and cross-sectional views. The cutting efficiency of the bur is then correlated with the angles of the bur and the percentage clearance space.

190. Temperature Developed in Rotating Dental Cutting Instruments. *D. C. Hudson, U. S. Air Force, National Bureau of Standards, Washington, D. C.* The Air Force has been interested in the problem of heat trauma in dental operating as a possible cause of pain occurring in teeth at high altitudes. Investigation was begun at the School of Aviation Medicine at Randolph Field, Texas, and has been continued at the National Bureau of Standards for the past two years. A direct approach to the problem of bur temperature measurement has been undertaken, placing a thermocouple in the head of the bur. Behavior of dental burs of steel and carbide has been studied at speeds up to 24,000 r.p.m. and data on temperatures of the burs have been compiled. Cutting efficiency at various speeds has also been determined and the data plotted as a function of time required to accomplish fixed depth cuts in a synthetic dentin-like material. Results obtained from a number of burs indicate that temperatures well above the limit of tolerance of tooth tissues to heat are attained by the cutting bur.

191. A Study of Cutting Efficiency and Vibration Production of Diamond Abrasive Cutting Instruments. *J. L. Hartley, U. S. Air Force, National Bureau of Standards, Washington, D. C.* One phase of the research on rotating dental cutting instruments which is being conducted in the National Bureau of Standards under the sponsorship of the USAF School of Aviation Medicine, Randolph Field, Texas, deals with the cutting efficiency and the vibration produced by diamond abrasive cutters. Diamond abrasive instruments of ten different manufacturers were evaluated from the standpoint of cutting efficiency. Speeds of rotation varied from 3,100 to 15,000 r.p.m. Vibration produced by these instruments was studied and photographed as displayed on the cathode ray oscilloscope. Amplitude and frequency of vibration are shown as functions of eccentricity and speed of rotation, respectively. Considerable variation exists in the behavior of diamond instruments of different manufacturers.

192. Design and Instrumentation of an Apparatus for Testing Dental Burs. *R. E. Jelinek, E. W. Skinner, R. L. Lindenmeyer, and R. L. Lasater, Northwestern University, Chicago, Ill.* A discussion will be given of the design and instrumentation of an apparatus for obtaining the relative cutting efficiency of dental burs under varied conditions of speed and feed. The design of the apparatus was based on the assumption that the relative cutting efficiency is a function of the rate of feed. Considerable effort was made to eliminate all extraneous variables and yet retain versatility, accuracy, and ease of operation. Special attention was given to the elimination of vibration in the driving mechanism. The object of the research is to study the effect of design on the cutting efficiency of the tools without reference to or influence of the dental handpiece.

193. Hydraulic Turbine Contra-Angle Handpiece. *R. J. Nelsen and C. J. Pelander, National Bureau of Standards, Washington, D. C.* A hydraulic turbine contra-angle handpiece which develops sufficient torque at speeds above 14,500 r.p.m. to cut dental enamel with diamond instruments has been designed. Two experimental contra-angle handpieces have been constructed of different turbine designs and with several types of bearings. The driving mechanism is a closed system; water flows to the turbine in the handpiece through nylon tubing from a hydraulic pump driven by ½ H.P. motor. This provides a power system relatively free of vibration for revolving cutting instruments.

194. Influence of Dental Materials Research on Dental Education. *M. K. Hine, Indiana University, Indianapolis, Ind.* An active dental materials research program in a dental school can be a vital force in improving the status of education. Research should develop new ideas, methods, and materials, of course, but its by-products (the stimulus it affords a teacher to keep informed and thus improve his teaching program, the personal satisfaction the individual receives from research, the direct and indirect effects on undergraduate and postgraduate students) amply justify the time, space, and money required by a research program. To improve the standards of materials used in dentistry the Council on Dental Research of the American Dental Association hopes to develop a cooperative program with dental schools and the Materials Section of the International Association for Dental Research to expedite and strengthen the program of developing specifications for dental materials.

195. Opportunities for Further Research and Development of Dental Materials. *W. T. Sweeney, National Bureau of Standards, Washington, D. C.* There is at the present time a great need for dental materials research of a fundamental nature. In the past almost all research in this field has been of the empirical type. The present standards are based on research on the materials which are in practical use by the profession of dentistry with the idea that only the superior ones will be acceptable. This is the best that can be done until more data are available on what is required for functional restorations. For example, denture resin standards are written around the physical and chemical properties of materials that have been used for dentures for a long time, originally rubber, and later acrylic resins; the values in general are not correlated with the forces and environment actually present in the oral application. This leads to a condition that may be too restrictive on certain properties because of the lack of knowledge of what is needed. The obvious answer is to make a study of the forces in typical cases to evaluate the properties required. Due to the fact that the replacement of lost teeth or parts of teeth with suitable materials occupies the majoriy of the dentist's time, it is essential that dental educators provide adequate instruction in the field of dental materials. This requires teachers trained in the physical sciences with a knowledge of dental applications. The future developments in research will best be served by close cooperation of the manufacturers, schools, and dentists in order that the manufacturer can better produce what the dentist needs and that the schools can teach the fundamental technics of the application of the products of research.

196. Specification Testing of Impression Compounds and Inlay Casting Waxes. *J. W. Stanford and G. C. Paffenbarger, National Bureau of Standards, Washington, D. C.* The most important physical requirements in American

Dental Association Specifications Nos. 3 and 4 for dental impression compound and dental inlay wax, respectively, are the flow or plasticity requirements. During recent testing of these materials considerable variation in the results obtained in different laboratories was encountered. It is believed that these differences may depend upon such factors as the temperature of the material when pouring the specimen, the temperature of the mold, the pressure applied and length of time the pressure is applied to the mold while the specimen is cooling, the age of the specimen before testing, the height of the water in the testing bath, and the position of the weight applied to the specimen while testing. In order to try to determine the effect of these factors, an exchange program was set up between the National Bureau of Standards laboratory and the laboratories of several manufacturers of impression compounds. The results of this program are not as yet complete, but it appears that the 40° C. testing temperature for flow is the point at which there is the most difficulty in obtaining agreement in results.

197. Forces Applied to Dental Materials During Mastication. *R. S. Ledley, National Bureau of Standards, Washington, D. C.* Restorative dental materials must withstand the functional forces applied during mastication. These functional forces arise from both the action of the food being chewed, and the reaction of the tissues supporting the restoration. Those factors that affect the forces due to food action are discussed in detail. The forces arising from supporting tissues depend on the character of the tissue; the reaction of hard tooth structure, periodontal membrane, and oral mucosa to the masticatory forces are considered. Thus, a knowledge of all forces acting on a restoration during function can be obtained; and with this information the strains induced in the restoration can be calculated. This type of program permits a critical evaluation of the physical properties of present-day restorative dental materials, as well as an evaluation of the effectiveness of various possible designs of restorations themselves.

198. Photoelasticity as a Research Technic for Dentistry. *D. B. Mahler, University of Michigan, Ann Arbor, Mich.* Photoelasticity is a method for determining the internal stresses within an arbitrarily shaped body subjected to an arbitrary force system. Because of the irregular shape of dental restorations and supporting tooth structures as well as the nature of the forces applied during mastication, this method of stress analysis is particularly useful for dental problems. A brief résumé of the theories of elasticity and plasticity, as well as that of photoelasticity, is given to provide a background for the understanding of this technic. Photographs are shown of a typical photoelastic setup and a description of its operation is given. Some typical dental problems which are applicable to this technic are also presented.

199. A Study of Amalgam Condensation Procedures With Emphasis Upon the Residual Mercury Content of the Increments. *M. J. Swartz and R. W. Phillips, Indiana University, Indianapolis, Ind.* The purpose of this investigation was to determine the physical properties of amalgam as related to the technic employed for removal of the excess mercury before and during condensation. Four technics were employed: (1) The "increasing dryness" procedure where progressively more mercury is removed from each portion of the mix before condensation. (2) Express all the mercury possible from each portion immediately before condensation of that segment. (3) Express all the mercury possible from the entire mix immediately; the amalgam is then continually softened by mulling in a squeeze cloth while the packing progresses. (4) Same as 3 but amalgam is not mulled. The physical properties tested

were dimensional change, flow, one and twenty-four hour compressive strength and residual mercury content. Both hand and mechanical condensation were employed. Results to date indicate little significant difference between the four procedures.

200. Metallography of Amalgam Alloys. *W. S. Crowell, S. S. White Manufacturing Co., Philadelphia, Pa.* The paper will discuss the teaching of these alloys and the metallography as affected by changing copper at a constant tin level and changing tin at a constant copper level.

201. Phase Changes in Silver-Tin Amalgams. *Gunnar Ryge, Marquette University, Milwaukee, Wis.* Crystals were produced experimentally on the surfaces of silver-tin alloys, representing the different phases of the silver-tin system, by contact with mercury. Crystals grown at mouth temperature were found to be larger than those grown at room temperature. Crystals were produced large enough for single crystal x-ray examination and certain phases were isolated and identified by this method. Selective etching technics will be discussed and demonstrated, and the setting theories of Gayler and Troiano will be discussed in the light of the observations of phase information.

202. Residual Mercury Determination Process. *W. H. Crawford and J. H. Larson, University of Minnesota, Minneapolis, Minn.* A mercury determination procedure has been developed to obtain a rapid measurement of residual mercury content of amalgams. The process involves the elimination of the mercury while protecting the other components from oxidation by maintaining an inert atmosphere. Since the physical properties of amalgams are related to their mercury content, and since an excess of residual mercury has a deleterious effect on these properties, this procedure is an aid in research attempts to obtain superior amalgam restorations, for it offers a quick, accurate (within 0.04 per cent) method for mercury determinations.

203. The Mechanism of Hygroscopic Expansion in Dental Casting Investments. *H. W. Lyon, Geo. Dickson, and I. C. Schoonover, National Bureau of Standards, Washington, D. C.* In the fabrication of dental gold castings the investment mold must be expanded to compensate for the shrinkage of the gold alloy on cooling from the liquid state to room temperature. One of the methods used to produce the necessary expansion consists of placing the mold in water during the setting period. Although a large amount of literature has been published on the hygroscopic technic, the mechanism of hygroscopic expansion has not been definitely established. On the basis of the data presented it is believed that hygroscopic expansion of dental investments is a continuation of the normal setting expansion during the period when the investment is in a semisolid state. The introduction of additional water causes hygroscopic expansion by permitting further hydration of the calcium sulphate during this time. The inert filler present in the investment serves to weaken its structure and thus permit the precipitating hydrate crystals to expand the mass of material.

204. Behavior and Measurement of Hygroscopic Expansion. *Karnal Asgarzadeh, University of Michigan, Ann Arbor, Mich.* An evaluation of the present methods of measuring hygroscopic expansion was made and it was found desirable to devise a method more consistent with the true over-all expansion. A volumetric measurement was designed which yielded a more consistent parameter. A four-sectioned inlay ring was made to allow for measure-

ments in the lateral direction, as well as to afford a constant minimum resistance to expansion in this direction. Using this volumetric measurement, the behavior of hygroscopic expanding investments was studied and it was found that the addition of a metered amount of water would consistently yield an expansion reproducible to within closer limits than any previous method. Also, the data derived from these volumetric measurements indicated behavior characteristics of hygroscopic expansion which heretofore have not been manifest.

205. Some Experiments on the Surface Hardness of Dental Stones. *E. W. Skinner and C. G. Gordon, Northwestern University, Chicago, Ill.* A number of treatments to increase the surface hardness of dental stone have been advocated. Some of them have been tried, but most have been found to be ineffective so far as could be determined by comparative values obtained with the Knoop indenter. Some increase in hardness was noted when the surface was treated in a solution of borax. The surface hardness of the stone is reduced when it is allowed to set against a hydrocolloid gel of the type used in commercial hydrocolloid impression materials, regardless of the previous treatment of the impression with various hardening solutions. There was no consistency in the results as to the type of hardening solution and its effect on different commercial hydrocolloids in their relation to the surface hardness of the stone, or vice versa.

206. Determination of the Residual Monomer in Methacrylate Resins. *N. S. Steck, Rohm and Haas Company.* A pyradine sulfate dibromide method of testing for unsaturated double bonds in both monomeric and polymeric acrylic materials is described. The results of testing a group of practical materials is presented as "residual monomer" analysis.

207. On Stresses, Crazing, and Distortion of Acrylic Resins. *C. W. Fairhurst and Gunnar Ryge, Marquette University, Milwaukee, Wis.* The occurrence of crazing and distortion in acrylic resin processed against tin foil substitutes was studied in an attempt to determine the extent and nature of internal stresses under various conditions. Stone models were made by duplicating a steel die. Specimens were processed against tin foil and tin foil substitutes in one or both of the halves of the mold. Crazing was studied and photographed from highly polished specimens (metallurgical procedure) using reflected and polarized light. The fit of the specimens was tested on the steel die immediately after curing, after storage, and after boiling in water. Crazing was produced in any acrylic surface processed against tin foil substitute. By two methods the crazing was found to exist to a depth of 2 to 3 mm. Crazing was intensified if the specimens were stored in air. Crazing was produced in specimens processed against tin foil after a few hours' storage in air. In specimens stored in air, then in water, crazing could not be produced. Specimens processed against tin foil substitutes on one or both surfaces showed distortion after storage in body temperature water and, particularly, after boiling. In all instances the warpage, or contraction, occurred toward the smoothest surface. The position of the flash did not determine the direction of the warpage. It is concluded that crazing and distortion are independent symptoms of internal stresses set up by friction between the acrylic resin and the surfaces of the mold during packing and polymerization.

208. Study of Clinical Behavior of Resins in Operative Dentistry. *B. Kornfeld, New York, N. Y.* Methacrylate polymer was mixed with powdered lead

glass before being combined with the monomer for polymerization. Small glass vials were filled with the resin thus treated. After polymerization the vials were immersed in barium sulphide solution at room temperature. Lead glass particles discolored wherever in contact with the barium sulphide. This test disclosed presence of leakage advancing from the exposed open surface of the vials to the base of the walls and eventually discoloring the base of the vial as well. A metal rod, passed through the walls of one vial, near its base, simulating an undercut for the purpose of keeping the filling material in place, retarded materially the advance of the discoloration beyond the retentive rod, but eventually the discoloration did reach the base of the sides and the floor of the vial. Cements and silicates did not show such release from the walls of the vials to permit the barium sulphide to enter the vial, or cavity, between the material and the wall of the vials. Though experiments in the mouth did reveal a discoloration of lead glass particles within the body of the resin restorations, the vial experiments showed discoloration about the walls and surface of the resins, but no deep penetration of the sulphide into the body of the material.

209. Adhesive Cements and Filling Materials. *F. A. Slack, Jr., University of Pennsylvania, Philadelphia, Pa.* This paper describes the physical and quantitative data supporting the adhesive characteristics of reinforced plastic cement. The physical characteristics of posterior filling materials are discussed.

210. A Study of the Electrical and Thermal Conductivity of Dental Cements. *Lloyd Phillips and R. W. Phillips, Indiana University, Indianapolis, Ind.* The purpose of this study was (1) to investigate the electrical and thermal conductivity of materials which are used at cement bases for restorative work and (2) to investigate the electrical and thermal conductivity of the restorative materials themselves. In determining the electrical conductivity, the galvanic cells which exist in the mouth due to dissimilar or similar metals and the currents that flow as a result of these cells are being duplicated in vitro. These cells are then utilized to investigate the insulating properties of various cementing materials. The electrical and thermal apparatus are explained, as well as the derivation of the formulas for calculating the resistance. Changes in the resistance pattern with such variables as thickness, surface area, composition, exposure to simulated mouth fluids, and time are being studied. In addition to electrical conductivity, the obtaining of thermal conductivity constants should together aid in the evaluation of these materials concerning their insulating ability as well as preference in restorative work.

211. The Plasticizing of Modeling Compound by Water. *A. R. Docking, Bureau of Dental Standards, Australia.* In the testing of modeling compound to the Australian Dental Standard, specimens for the plastic deformation tests have been prepared by kneading the compound and pressing it into the mold. For the American Dental Association specification cast specimens are used. The investigation described was undertaken because of two facts noted when the Australian method of preparing specimens was used. (1) The plastic deformation figures obtained on certain compounds in the United States list of certified materials were found to be consistently higher than the upper limit allowed by the A.D.A. specification. (2) Changing from one operator to another produced widely divergent results. It was shown that the incorporation of water into the compound plasticized it to such an extent that the plastic deformation and impression-taking ability tests were seriously affected.

The compounds differed greatly in their sensitivity to the different methods of preparation. Simple experiments demonstrating the role of water and a method of kneading to give uniform results are described.

212. Disintegration of Solder Joints of Base Metals. *S. M. Bien, New York, N. Y.* Twelve joints were soldered with each of three types of solder. Only a 400 fine white gold solder withstood the treatment imposed without deterioration.

VII. Executive Proceedings and Register of Newly Elected Officers and Members

A. COUNCIL ACTIVITIES

Activities Between General Meetings

Between March 21, 1952, and March 20, 1953, the Council approved the details of the announced budget disbursement items for the operation of the officers of the Secretary-Treasurer and Assistant Secretary-Treasurer for the 1952-1953 Association year, and nominated candidates for the general officers of the Association, these candidates to appear on the official ballot as the Council candidates.

Council Meetings, March 20 and March 22

At both sessions of the Council the reported acts of the Council were effected only after due discussion and formal votes.

Council members present at first session, with President Hine presiding, were: Arnim, Arnold, Bibby, Burrill, Costich, Crawford, Dobbs, Grossman, Hall, Hatton, Hill, Hine, Jay, Kitchin, Kreshover, Levy, Manly, Noyes, Oartel Orland, Paffenbarger, and Robinson. The acts and recommendations to the Executive Proceedings of this session follow: (a) The proposed plan to hold two or more meetings each year was opposed. (b) A three-day meeting in 1954 was approved. (The time: the three days preceding the meeting of the American Association of Dental Schools.) (c) The Council members opposed relinquishing the right to use the time for meeting purposes on Sunday night, but advised the Association to schedule no Sunday night scientific session in 1954. (d) The proposal to advise the listing of essential degrees of program participants was not approved. (e) The following schedule was approved for the preliminary announcements for the 1954 meeting in the April issue of the *Journal of Dental Research; second,* September 15; *third,* about October 15. (f) Set the closing date for the receipt of items for the 1954 program at *December 15, 1953.* (g) The Council advised the inclusion of the announcement of the Dental Science and Dental Art 1954 Novice Award, also a statement with reference to publicity releases before the meeting and what constitutes desirable practice in instances of dual and multiple authorship in the oral presentation portion of the program, in early preliminary announcements. (h) The Program Committee was advised to have the program printed about January 15 in order to secure early mailing of the program to members and participants.

Council members present at the second session, March 22, President Hine in the chair: Arnold, Bibby, Brashear, Burrill, Dobbs, Hall, Hatton, Hill, Hine, Oartel, Orland, Robinson, Swanson. This group received, approved, and ordered audits of the 1952 calendar year financial operations reported by the Secretary-Treasurer as follows:

Receipts

Paid to D. Y. Burrill	$3,449.16	
Paid to E. H. Hatton	709.71	
From Mosby Co. (Gratuities)	1,800.00	
Interest	32.50	
Collected and refunded	96.00	
Total		$6,087.37

Disbursements

Postage	$ 112.70	
Clerical	316.41	
Printing and supplies	335.73	
Telephone and telegraph	11.65	
Meeting expenses, etc.	50.00	
Unexpended petty cash	50.00	
Publication Committee Gratuities	1,250.00	
E. H. Hatton, Expense Gratuity	158.81	
C. V. Mosby Co. JDR subscriptions	3,402.00	
Total		$5,853.30
1952 Surplus		$ 234.07

The following disbursement budget was approved for the calendar year of 1954:

Secretary-Treasurer's offices	$1,000.00	
Gratuities, Publication Committee	1,800.00	
Block JDR subscriptions	4,500.00	
Total		$7,300.00

The budget is based on estimated income and details to be approved by mail vote of Council. The dues assessment for the 1954 calendar year was set at the rate of $9.00 per member, to be divided on the basis of $2.00 for the general fund and $7.00 for the *Journal of Dental Research* fund from each $9.00 collection. The remission of the dues assessment for the 1954 calendar year was ordered for all members residing outside of North America. The submission of an amendment to the constitution covering Article III on membership was approved (these changes are printed elsewhere in these proceedings). M. M. Krogman was reelected to membership. A list of candidates to be submitted to the Association for election to membership was approved. (This list is printed elsewhere in these proceedings.) The organization of new sections in Norway and Helsinki, Finland, was approved. The inactivation of the Prague and Budapest sections was approved.

B. REPORT OF EDITOR—1953

Volume 31 of the *Journal of Dental Research* included ninety-five original papers. The rate of publication failed to keep up with the rate of receipt of papers. During 1952 the editor received 133 papers, of which 126 were accepted. The number of pages in each volume was increased to almost 900, but the *Journal* does not meet the demands.

During 1952 seventy-one pages were used for proceedings of the Annual Meetings, and the proceedings of three sections took an additional five pages. We are of the opinion that these proceedings, and the abstracts therein, are

valuable contributions to dental literature. An additional ninety-three pages were devoted to a biographical directory of IADR members. This, too, we believe a worthy project which should be repeated at intervals of two, three, or five years.

There are several means of making the contributions and the published material more nearly equitable year by year. The editor could be more critical in accepting papers, but by doing so might deny publication to potentially important work. The editor could shorten papers by stricter editorial rephrasing, but such practice appears to interfere with the inherent rights of the author. The authors could write in briefer (and often clearer) manner. The editor does return many accepted papers for such revision, but verbosity, in a mild way, seems to be a fault of too many investigators. The number of pages in the *Journal* could be increased. This would be ideal but impractical.

The C. V. Mosby Company reports that its losses on the *Journal* in 1952 were approximately $2,900. The small number of paid subscribers (less than 3,000) and the dirth of advertising lead to this unfortunate state of affairs. The publishers have recommended that the subscription price be increased to members and non-members by $1.00 each per year. The number of subscribers should be increased and cards have been prepared for distribution by members in a subscription campaign. Those members who are in position to do so should encourage the placement of advertisements in the *Journal*. The Association has a moral obligation to support its *Journal* and to increase its influence.

A representative of the Scandinavian Dental Schools has approached us concerning the possibility of a supplement, or portion of the *Journal*, being devoted to papers prepared in the Scandinavian countries and to some informational material on dental education. This requires our careful consideration.

The editor wishes to thank all those who have assisted with Volume 31, including Dr. E. H. Hatton, Dr. Harold C. Hodge, Dr. Paul C. Kitchin, Dr. J. B. Brown, and others, including the contributors.

HAMILTON B. G. ROBINSON, *Editor.*

C. EXECUTIVE SESSIONS

First Session, Friday Morning, March 20, 1953

The meeting was opened by President Hine with a few appropriate remarks, after which he called for the minutes of the 1952 general meeting. Secretary-Treasurer Hatton reported that the minutes and proceedings had been printed in the August issue of the *Journal of Dental Research* and moved that they be approved as printed, but with one correction which follows: On page 524 of the proceedings after the words "Harwell, Atomic Energy Establishment," insert the name *James Thewlis.* This motion was seconded and carried. It was also reported at this time that the name of *Myer Klatsky* of New York had been accidentally omitted from the biographical directory member list as printed in the October, 1952, issue of the *Journal.* Frederic James and Louis Grossman were appointed tellers to receive and count the ballots for the election of officers. Brief reports were received from the Program and Novice Award Committees. The session was adjourned to Sunday afternoon.

Session of the North American Divisions, Sunday Morning, March 22

R. W. Bunting presided. A report was made of the recent holiday session at the last meeting of the A. A. A. S., and a request was made for full cooperation in the plans to promote the coming meeting with suggestions for the program, etc. Adjourned.

Closing Session, Sunday Afternoon, March 22, 1953

President M. K. Hine presided. The tellers reported that 344 ballots had been cast and that all of the Council candidates had been elected by substantial and nearly unanimous majorities. (The list is printed elsewhere in these proceedings.) However, H. A. Hunter polled 78 votes for trustee. Dr. Harold Hodge for the Committee on the Dental Science and Dental Art Award reported that the Committee had voted unanimously to name John J. Sallery of Rochester as the winner, and then very graciously made the presentation. The Committee named J. L. Hartley, U. S. A. F.; M. M. Matt, Western Reserve University; and K. J. Paynter, University of Toronto, as being entitled to honorable mention in the contest. It was moved and seconded that all the acts and proceedings of the Council be ratified and approved as read, including approval of the 1954 budget and dues assessment of $9.00 per dues-paying member; this motion was carried without any opposition. The proposed amendment of Article III of the constitution in the form discussed by the Council and approved by them was introduced, and its submission to the members was authorized by a large majority.

The list of persons nominated for membership as approved by the Council and by special motion (rules suspended unanimously) as qualified for membership was read. It was moved and seconded that the rules be suspended and a single ballot be cast by the secretary-treasurer for the election of these nominees as members. This motion was carried without a dissenting vote or any objection, and the white ballot for election was formally cast. (This list is published elsewhere in these proceedings.) By motion, the State Bank and Trust Company of Evanston, Ill., and the Lincoln Bank and Trust Co., of Louisville, Ky., were approved for the banking uses of the Association, and F. A. Arnold, Jr., George C. Paffenbarger, and Edward H. Hatton were authorized to sign checks and orders for disbursement of the Association's funds, but with signatures of any two on each check or order. The Council was authorized to act for the Association between meetings and to name two honorary vice-presidents. A motion of appreciation and thanks to the local committee and many others was passed very enthusiastically, selecting for personal mention Drs. Boyle, Grossman, James, Wilde, and Williams, as well as Mr. Bennett of the hotel, the local optical companies who loaned the splendid projectors, and the company of helpful and courteous students. Also, a special vote of thanks to the Chamber of Commerce was passed.

At this point and just before his concluding remarks, President Hine ordered that the list of the members who had passed away since the last meeting be read, and that these members be honored silently by a standing audience. (This list is printed elsewhere in the proceedings.)

The newly elected officers (as printed elsewhere in these proceedings) were called to the space before the president and properly installed and inducted into their respective offices. Dr. Arnold then delivered his inaugural address. Opportunity was given the secretary-treasurer to remind the Association that retiring President Hine had given the Association three years of outstanding and constructive service which had been especially appreciated by the officers with whom he had worked, as well as by other members of the Association, and which should be recognized by all.

There being no other business, President Arnold adjourned the 1953 general meeting.

VIII. PROPOSED CHANGE IN THE CONSTITUTION

The following changes in the constitution are proposed and urged for adoption in order to materially broaden the efforts of the Association to attain its ob-

jectives as stated in Article II, and specifically in portion (b) "to encourage and facilitate cooperative effort and achievement—by investigators in all nations and in every division of stomatology."

Submitted by Harold C. Hodge, Paul C. Kitchin, Paul E. Boyle, J. L. T. Appleton, and J. R. Blayney.

All of Article III. Membership, is hereby repealed and replaced with a new and substituted Article III, the text of which follows herewith:

ARTICLE III. MEMBERSHIP

Section 1. *Kinds.* There shall be two classes of membership: (a) active, and (b) associate.

Section 2. *Active Members.* (A) Eligibility. Any person who has conducted and published an account of a meritorious original investigation in dental science, or in any of the sciences contributory to oral health care, is eligible to active membership in this Association, provided such person conforms to the recognized standards of professional ethics.

(B) Nomination. (a) Nominations to active membership may be presented to the Council at any time, for consideration at the succeeding annual meeting of the Association. (b) The Council shall promptly determine, for each nomination to active membership in the Association, whether the nominee meets the requirements of eligibility, and at the first succeeding annual meeting shall report to the Association the findings relating to each eligible nominee.

(C) Election. (a) Election of new active members may occur only at annual meetings. (b) Election to active membership shall be by ballot. A two-thirds affirmative vote of those present and voting shall be necessary for election to active membership.

(D) Initiation. Initiation of active membership requires delivery to the secretary-treasurer of a signed acceptance of election thereto.

Section 3. *Associate Members.* (A) Qualifications. Any person willing to support the objectives of this Association and whose formal nomination is sponsored in writing by three active members is eligible for associate membership.

(B) Election. Election of new associate members may occur only at annual meetings of the Association and shall be by ballot. A two-thirds vote of those present and voting shall be necessary for election.

(C) Initiation. Initiation of membership requires delivery to the secretary-treasurer of a signed acceptance of membership thereto.

(D) Rights and privileges. Associate members shall have all the rights and privileges of membership except that of voting.

Section 4. *Discontinuance of membership.* (A) Membership (active or associate) may be automatically terminated, by a member, by delivery of a formal notice to the secretary-treasurer of that member's resignation.

(B) Membership may be automatically discontinued, for any member, by delivery to the member of a formal notice from the secretary-treasurer of the decision, at an annual meeting of the Association by a two-thirds vote of those present and voting, that the member's association has been terminated.

The Article III of the constitution of the INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH as reproduced immediately following this introduction is repealed and inactivated as proposed above.

Article III. Membership

SECTION 1. *Eligibility.* Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory

to oral health-care, is eligible to membership in the Association, provided such person conforms to the recognized standards of professional ethics.

SECTION 2. *Nomination.* (A) Nominations to membership may be presented to the Council at any time, for consideration at the succeeding annual meeting of the Association.

(B) The Council shall promptly determine, for each nomination, whether the nominee meets the requirements of eligibility, and at the first succeeding annual meeting shall report to the Association the findings relating to each eligible nominee.

SECTION 3. *Election.* (A) Election of new members may occur only at annual meetings of the Association.

(B) Election to membership shall be by ballot. A two-thirds affirmative vote of those present and voting shall be necessary for election.

SECTION 4. *Initiation.* Initiation of membership requires delivery to the Secretary of a signed acceptance of membership thereto.

SECTION 5. *Discontinuance.* (A) Membership may be automatically terminated, by a member, by delivery of a formal notice to the Secretary-treasurer of that member's resignation.

(B) Membership may be automatically discontinued, for any member, by delivery to the member of a formal notice from the Secretary-treasurer of the decision, at an annual meeting of the Association by a two-thirds vote of those present and voting, that the member's association has been terminated.

IX. OFFICERS AND MEMBERS ELECTED

ACTIVE OFFICERS: President—*Francis A. Arnold, Jr.,* National Institute of Dental Research. President-elect—*George C. Paffenbarger,* National Bureau of Standards. Vice-president—*Paul E. Boyle,* University of Pennsylvania. Secretary-treasurer—*Edward H. Hatton,* Northwestern University. Assistant Secretary-treasurer—*Dan Y. Burrill,* University of Louisville. Trustee for a five-year term—*H. Trendley Dean,* National Institute of Dental Research.

MEMBERS ELECTED. FROM NORTH AMERICA: Alabama—*J. A. Gibilesco,* University of Alabama. California—*L. M. Frandsen* and *Howard W. Myers,* University of California. District of Columbia—*Phillip Person,* Army Medical Center. *Gerhard M. Bauer* and *B. K. Forscher,* Georgetown University. *Alphonse Forziatti, Aaron S. Posner, Andrew F. Serio,* and *John W. Stanford,* National Bureau of Standards. Georgia—*John Haldi* and *Winfrey Winn,* Emory University. Illinois—*J. Roy Doty,* American Dental Association. *William J. Carter, Lewis H. Daniels,* and *Walter L. White,* Great Lakes Naval Training Station. *Dorothy Rizzo DeRose* and *T. M. Graber,* Northwestern University. *Helen Kutozov* and *Leo M. Sreebny,* University of Illinois. Maryland—*R. J. Fitzgerald* and *Rachael Harris,* National Institute of Dental Research. *M. F. Aisenberg, Jr.,* University of Maryland. Michigan—*Edwin Henry,* University of Michigan. Minnesota—*Hans Muhlemann* and *Leon Singer,* University of Minnesota. Missouri—*Duane F. Taylor,* Washington University. New York—*Nicholas A. DiSalvo* and *Wesley Halpert,* Columbia University. *Morris B. Auerbach, J. E. Fiasconaro,* and *H. I. Jahoun,* New York University. *Erling Johansen,* University of Rochester. *Alexander Seelig,* Montefiore Hospital. Ohio—*William G. Sprague,* Western Reserve University. Pennsylvania—*Paul W. Montgomery,* U. S. Army, Phoenixville. Virginia—*Harold W. Syrop,* Medical College of Virginia.

Canada, Ontario—*Egil P. Harvold* and *Robert E. Moyers,* University of Toronto. Total from North America, 39.

MEMBERS ELECTED FROM COUNTRIES OUTSIDE OF NORTH AMERICA: Denmark—*E. Husted,* University of Denmark. England—*B. E. D. Cooke,* Guy's Hospital and *C. H. Tonge,* Sutherland Dental School. Finland—*Yrjö V. Paatero* and *Mauri Pohoto,* University of Finland.

Norway—*Thorwald Kvam*, Trondheim. *Birger Nygard, Kaare Reitan, Harald F. Sand, Reidar Selmer-Olsen* and *Jens Waerhaug*, University of Norway, Oslo. Sao Paulo (South America)—*Francisco Degni* and *V. P. Delgado*. Total outside North America, 13.

New Member Total, 52.

RESIGNATIONS DURING THE 1952-1953 YEAR: David D. Bloom, Boston; William Randall, Louisville; Houghton Holliday, New York; R. F. Nagle, Boston; A. F. Treloar, Minneapolis; Betty Jane White, Cleveland.

NECROLOGY. *Marguerite T. Dean*, Memphis, School of Dentistry, University of Tennessee. *Theodore Kaletsky*, Jamaica, N. Y. *James Nuckolls*, San Francisco, School of Dentistry, University of California. *N. C. Ochsenhirt*, Pittsburg, School of Dentistry, University of Pittsburg. *J. A. Sinclair*, Asheville, North Carolina. *Ernest G. Sloman*, San Francisco, College of Physicians and Surgeons. *Charles M. Waldo*, Boston, Harvard School of Dental Medicine.

MEMBERS IN ATTENDANCE AT ONE OR MORE SESSIONS, LISTED BY SECTIONS

UNITED STATES

Ann Arbor—Bunting, Crowley, Jay, Kerr, Peyton, Ramjford. *Baltimore*—Dobbs, Hahn, McCrea. *Birmingham*—Finn, Klapper, Pigman, Volker, Wuehrmann. *Boston*—Barr, Berk, Cohen, Dunning, Glickman, Goldman, Greep, Keyes, Kraus, Lisanti, Marjerison, Manly, Marshall-Day, Anna Morse, Munson, Nizel, Shaw, Shiere, Sognnaes, Turesky, Turner, Yurkstas. *Chicago*—Bhaskar, R. Q. Blackwell, Blayney, Brodie, Burstone, Calandra, E. D. Coolidge, T. B. Coolidge, Engel, Fosdick, Freeman, Hatton, Hill, Kesel, Martin, Molnar, Orland, Pearlman, Romnes, Rovelstad, Schour, Sicher, Skinner, Teuscher, Tilden, Tylman, Wach, Wainwright, Wallace, Weinmann, Wentz. *Cleveland*—Bruckner, Burwasser, Chase, Gilkison, Gillis, Hill, Kimball, Wylie. *Columbus*—Kitchin, Permar, Robinson, Spangenburg. *Houston*—Arnim. *Indianapolis*—D. A. Boyd, Hall, Hine, McDonald, Muhler, Phillips, Shafer, Van Huysen. *Iowa City*—Simon, Smith, Wessels. *Kansas City*—Moore, Warner. *Louisville*—Burrill, Myers, Wolfe. *Minnesota*—Crawford, Mitchell, Stafne, Zander. *Nebraska*—Hooper, Keys, Waggener. *New York*—Bartelstone, Beube, Bevelander, Bien, Bodecker, Chilton, Ellison, Goldsmith, Horowitz, Klein, Lefkowitz, Levy, Miller, F. W. Morse, Neumann, Neuwirth, Seidler, Siegel, Singer, Sobel, Stahl, Stein, Tanchester, Trautz, Waugh. *Pacific Northwest*—Noyes, Savara, Snyder, Thomas. *Philadelphia*—Bender, Boyle, Burkett, Crowell, Gore, Grossman, McClendon, Moore, Prinz, Rothner, Seltzer, Taylor, Wilde, Williams. *Pittsburgh*—Cox, Leung, Nathans, Strader, Swanson. *Richmond*—Brashear, Clough, Coy, Knighton, Kreshover, Lyons. *Rochester*—Afonsky, Bibby, Brudevold, Costich, Gilda, Hein, Hodge. *St. Louis*—Allen, Boling, Brandhorst, Dietz, Gurley, Rosebury. *San Francisco*—Agnew, Baume, Leicester, Scrivener, Thomassen. *Southern California*—Bavetta, Bernick. *Tennessee*—Ginn. *Washington*—Arnold, Burnett, Caul, Dickson, East, Fritz, Goldberg, Hampp, Hess, Kaplan, Knutson, Kruger, Likens, Ludwick, McBride, McClure, Medak, Nelsen, Oartel, Paffenbarger, Parke, Rault, Rogosa, Schoonover, Scott, D. L. Smith, Souder, Stephan, R. W. Taylor, Zipkin.

Not Members of Sections—Brauer, Brumfield, Fischer, Fleming, Henry, Hunt, Luster, Mandel, Nelson, Ryge, Savchuck, Wessinger, Wise.

CANADA

Toronto—Ellis, Hunter, Nikiforuk, Rae.

FOREIGN

Bevilacqua, Iusem, Reichborn-Kjennerud, Toverud. Total, 229.

Total members, 229. Guests, 352. Combined Attendance, 581.

MEMBERS VOTING AT THE ANNUAL ELECTION

Afonsky, Agnew, Aisenberg, G. M. Anderson, Appleton, Armstrong, Arnold, Artageyvetia-Allonde, Backer-Dirks, Bagnall, Bailey, Bauer, Baume, Bay, Becks, Bender, Bernick, Bernier,

Beube, Bevilacqua, Bhaskar, Bibby, Bier, Blackerby, R. E. Blackwell, Bloom, Blumenthal, Boucher, Boulger, T. M. Boyd, Bradel, Brandhorst, Brashear, Breitner, Broadbent, Brodie, Brodsky, Broughton, Brudevold, Brumfield, Bryan, Burket, Burnett, Burrill, Camalier, Caul, Carr, Chaves, Cheyne, Chilton, Chumbley, Clark, M. M. Cohen, E. D. Coolidge, Costich, Cox, Coy, H. M. Crawford, W. H. Crawford, Cross, Crowley, Dahlberg, Dale, Darling, Darlington, Davis, Dawson, Dean, Dickson, Dietz, Dobbs, Docking, Dragiff, Dreizen, Dressel, Dummett, J. M. Dunning, Easlik, East, Easton, Eggers-Lura, M. D. Elliott, Ellis, Emslie, English, Ennever, I. A. Epstein, Sidney Epstein, Erikson, Everett, Ferguson, Figg, Finn, Fischer, Fisher, Fleming, Fox, Freeman, Frey, Fritz, Gafafer, Gans, Gilda, Gilkison, Gilmore, Ginn, Glass, Glasson, H. J. V. Goldberg, Goldhaber, Goldman, Goldsmith, Goodsell, Greep, Grosby, Grossman, Gruebbel, Gurley, Hadjimarkos, Hahn, Hansen, Hardwick, Harrison, Hatch, Hatton, Healey, Hein, Hess, Higley, Hildebrand, I. N. Hill, T. J. Hill, Hine, Hockett, Hodge, Holst, Hooper, Horowitz, Hubbell, Hunt, Hunter, Inskipp, Iusem, Jacobson, James, Jasper, Jay, Johnson, Jordan, Kaiser, Kaplan, Keilty, Kerr, Kesel, Keyes, D. A. Keys, Khalifah, Kitchin, Klaffenbach, Klapper, Klein, Kniesner, Knighton, Knudtzon, Knutson, Koski, Kramer, Krasnow, Kraus, Kreshover, Krogh, Krogh-Poulsen, Kruger, Kurth, Land, Lasater, Law, Lefkowitz, Leung, Levy, Lewin-Epstein, Lieban, Lieber, Lisanti, Lobene, Lovestedt, Lowery, Ludwick, Lund, Luster, Lynch, MacDonald, McCauley, McClure, McCrea, Maier, Main, Manly, Marshall-Day, Martin, Mason, Massler, Medak, Meisel, Mello, Mercado, Merkeley, Merritt, S. C. Miller, Millhon, Miner, Mitchell, Moen, N. A. Moore, T. E. Moore, A. L. Morse, F. W. Morse, A. H. Mueller, Munson, R. E. Myers, Nathans, Nelsen, Nelson, Neumann, Nikiforuk, Nizel, Oartel, Ockersee, Olech, C. P. Oliver, O. A. Oliver, Orland, Ostrom, Paffenbarger, Palazzi, H. B. Palmer, Parke, Patterson, Pearlman, Pedersen, Permar, Peyton, Pffeiffer, Phatak, Phillips, Pigman, Pindborg, Podolin, Prinz, Rabinowitch, Radusch, Rae, Ramjford, Rault, Reichborn-Kjennerud, Revesez, Ritchey, H. B. G. Robinson, Rosebury, Rosenstein, Rovelstad, Rushton, Russell, Ruzicka, Ryder, Ryge, Sarnat, Sartori, Savara, Savchuck, Schoonover, Schour, Schroff, D. B. Scott, Scrivener, Seidler, Seltzer, Shafer, J. H. Shaw, J. C. M. Shaw, Shell, Sherman, Schiere, Sicher, Simmonds, W. J. Simon, C. A. H. Smith, D. L. Smith, E. L. Smith, Smyd, Snyder, Sobel, Sognnaes, Sorrin, Spangenburg, Speidel, Stack, Stafne, Staz, Steadman, Steinmeyer, Stephan, G. G. Stewart, Stones, Stout, Suher, H. A. Swanson, W. F. Swanson, Swartz, Sweet, Taber, Tainter, R. W. Taylor, Telford, Teuscher, Thoma, B. O. A. Thomas, J. R. Thompson, Thomson, Tiecke, Trautz, Ussing, Van Huysen, Volland, Waggener, Wainwright, Walter, Ward, Warner, Waugh, Weber, Weinmann, Weisengreen, Wessinger, Wheatcroft, Wheeler, Wilbur, Willman, Wolcott, W. R. Wolfe, Jr., Wright, Young, Zander, Zipkin. Total, 344.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section

School of Dental and Oral Surgery, Columbia University, Jan. 29, 1953

Compiled by Frances Krasnow, *Editor, New York Section*

1. Porosity of Root Canal Dentin. *Maurice Buchbinder, New York, N. Y.* It has been demonstrated that the root canal dentin after pulp removal may be deeply stained by dyes and metallic salts. The amount of absorption has not been studied on a quantitative basis, although much interest attaches to this since a porous dentin may be a medium for the retention of toxic material. The root canal dentin was investigated for porosity both in vivo and in vitro using a radiographic method. Saturated calcium and sodium iodide were sealed into canals of teeth under endodontic treatment and also into extracted teeth as controls. The iodides are x-ray visible but they disappear in time by diffusion into the dentin of the canal. Necessary controls were used which show that (1) the iodides are not volatile and are stable; (2) they are not diffused into the periapical tissues; (3) they do not leak back into the mouth through the seal; and (4) they diffuse into the porous dentin of the canal walls. Radiographic series were made which prove that the dentin of the canal is capable of absorbing and holding very large amounts of soluble, diffusible material. A direct application to endodontic practice consists in realization that the root canal walls may contain large doses of toxic protein fraction and bacterial toxins absorbed into the walls. Those may well be a factor in etiology and therapy of periapical lesions.

2. The Effect of Penicillin G Potassium Plus Calcium Carbonate Upon Surgically Exposed Dental Pulps of a Rhesus Monkey. *Alexander Seelig, Richard C. Fowler,* and David Tanchester, Dental Department, Montefiore Hospital, New York.* Published in Proceedings of the 31st General Meeting of the I. A. D. R., *J. D. Res.* **32**: 683, 1953.

3. Odontogenesis of the Rat Molar. Prenatal Stage. *William Lefkowitz, Charles F. Bodecker, and Dorothy Mardfin,* Dental Department, Montefiore Hospital, New York.* The rat molar lends itself in embryological studies because it is possible to obtain embryos of known age. It has been used extensively in experimental studies in the field of transplantation and in vitro investigations. A prerequisite to evaluating developmental changes requires a chronological table of normal growth and development. One hundred fifty-six embryo heads of known age were sectioned. Odontogenesis was observed at 24-hour intervals from the first epithelial invagination to birth. The dynamics of odontogenesis and the cytomorphosis and function of each element of the tooth germ is described. The observations have established new concepts of the function of the elements of the tooth germ.

4. A Histochemical Study of the Development of Dentin. *Percy L. Johnson and Gerrit Bevelander, New York University College of Dentistry, Department of Histology, New York, N. Y.* The observations to be reported are

*Guest.

based on histochemical studies of developing dentin with special reference to the following substances: (1) Ribonucleic acid, found in developing odontoblasts, increases in amount as cell becomes functional. (2) The ground substance of the pulp, basement membrane, Korff's fibers and odontoblasts are Hotchkiss-positive, indicating the presence of neutral polysaccharides in these structures. Uncalcified dentin is weakly Hotchkiss-positive; the tinctorial property of dentin is enhanced as calcification becomes more pronounced. (3) Acid polysaccharides as indicated by metachromasia are likewise present in the ground substance of the pulp, and in uncalcified dentin. The formation of calcospherites gradually displaces this metachromatic ground substance. When the dentin appears to be partially but uniformly calcified, this tissue becomes basophilic in character. Later the more highly calcified dentin loses its basophilic characteristic. These observations indicate that ribonucleic acid increases as the odontoblast becomes functional; mucopolysaccharides appear to be more abundant in calcified than in noncalcified dentin. Metachromasia indicative of acid polysaccharides is evident in uncalcified dentin. The orthochromatic and nonstaining portions of calcified dentin may be indicative of the absence of acid polysaccharides or polysaccharides present in concentrations which do not respond to staining metachromatically.

5. Stripping Emulsion Radioautography of I-131 Uptake in Human Teeth (In Vitro). *Herbert J. Bartelstone and Henry Wolff,* National Institute of Dental Research, National Institute of Health, U. S. Public Health Service Hospital, Federal Security Agency, Staten Island, N. Y.* Published in Proceedings of the 31st General Meeting of the I. A. D. R., *J. D. Res.* **32**: 636, 1953.

6. Measurement of Enamel Radioactivity Within the Mouth. *H. H. Neumann and N. A. DiSalvo, School of Dental and Oral Surgery, Columbia University, New York, N. Y.* Measurements were carried out with a small Geiger-Mueller tube instrument, small enough to be introduced into the human mouth or the mouth of a rhesus monkey. The very thin end window of the tube is smaller than the crown of one single molar and beta radiation emitted from one tooth can be measured. Sufficient shielding of the background can be achieved to obtain varying differential counts from different teeth within the mouth. This Geiger-Mueller counter is a convenient instrument to follow the trend and rate of excretion of phosphorus from day to day and week to week from the enamel of single teeth without necessity of their extraction. The counter has a probe section of only one-fourth of an inch diameter and is well suited to beta ray counting. We injected 500 microcuries of P-32 intraperitoneally into rhesus monkeys and obtained high counts on single molar teeth. After a unilateral trigeminal nerve resection of the third branch, including the motor fibers, the counts obtained from molars on the normal and the operated side were unequal. The differences were highly significant, in the order of two to three times over background, and were recorded repeatedly. Control animals showed an equal rate of emissions from right and left molars. We are unable to explain at this time the apparent large differences in the radioactivity of the enamel on the functioning and the nonfunctioning half dentitions.

7. Effects of Strontium and Fluoride on Bone Repair. *William B. Savchuck, National Institute of Dental Research, U. S. Public Health Service Hospital, Staten Island, N. Y.* Published in the Proceedings of the 31st General Meeting of I. A. D. R., *J. D. Res.* **32**: 680, 1953.

*Guest.

8. The Topology of the Human Premaxilla. *Charles R. Noback* and Melvin L. Moss, Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.* The persistence of the labial lamina of the premaxillary bone in the human adult remains a confused problem for both anatomists and physical anthropologists. A critical review of the literature leads to the probable conclusion that the labial lamina of the human premaxilla persists in the adult without operculation by the maxilla. This review of the pertinent data may help bring the question nearer to clarification in the minds of those dealing with its implications. We must consider morphogenesis in addition to morphology. The methodology is properly embryological and not comparative. Two key points must be observed. The first is that no material younger than 6 to 7 weeks, nor older than 9 weeks (fetal age) may be properly utilized, the onset of ossification occurring at the former and the union with the maxilla at the latter. Any studies on later material will necessarily miss this key stage and so invalidate itself. The second key point is terminological. Confusion exists between the terms "osseous process" and "embryonic masses" of the branchial arch region, also termed "process." This has led to confusing the contribution which the embryonic maxillary process makes to the formation of the premaxilla with the existence of an osseous maxillary process labially covering the premaxilla in the adult. A further confusion exists between the contributions of the maxillary process to the soft tissue of the upper lip and those to the osseous structure itself.

*Guest.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

SCIENTIFIC PROCEEDINGS OF THE SAN FRANCISCO SECTION, FEB. 19, 1953.

Compiled by LOREN B. TABER, *Editor, San Francisco Section*

1. THE ROLE OF PHOSPHATASE INHIBITORS IN DENTAL CARIES. *Paul R. Thomassen, College of Physicians and Surgeons, San Francisco, Calif.* Studies with radioactive cadmium indicate that there is very little uptake of this substance by the teeth during their formation, but when the cadmium is administered over a long period of time in drinking water, an absorbed layer of cadmium is found on the surface of the molars of rats. This may have some effect either in inhibiting or enhancing caries activity, depending on concentration. Beryllium is a more active inhibitor of alkaline phosphatase than of acid. Its action in decreasing acid production by lactobacillus acidophilus depends on its concentration. In general, it is more active at the concentration at which inhibition of acid phosphatase begins.

2. EXPERIMENTAL PROCEDURES USING RADIOACTIVE ISOTOPES IN PHOSPHATASE STUDIES. *Leland W. Nicholas and George J. Denzler, College of Physicians and Surgeons, San Francisco, Calif.* The practical details of using rats and hamsters and of handling radioactive material in such studies were described.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the Philadelphia Section
University of Pennsylvania School of Dentistry, Feb. 16, 1953*

Compiled by Neal W. Chilton, *Editor, Philadelphia Section*

1. A Study of a Biopsy Service: Problems in Diagnosis of 1,283 Cases. *Helen McF. Woodbridge, University of Pennsylvania, Philadelphia, Pa.* This study was undertaken to evaluate the variety and types of lesions of the mouth and face with special reference to incidence of neoplasms and cancers. The series ran from January, 1947 to May, 1952, and consisted of about 1,500 tissue specimens, of which one-fifth were discarded as inappropriate. A working classification was adopted and the various categories were discussed. Difficulties in accurate diagnosis are explained. Lesions considered to be benign neoplasms numbered 33; semimalignant neoplasms, including mixed tumors, ameloblastomas, and adnexal (basal cell) carcinomas, 13; malignant tumors, 42, of which 4 were sarcomas, 34 epidermoid carcinomas, and 4 adenocarcinomas. Microscopic examination of tissue sections was considered in relation to clinical diagnosis of pathologic conditions, and prognosis for the patient.

2. Some Developments in Self-Curing Resins. *John F. Glenn, L. D. Caulk Company, Milford, Del.* Seven groups of nitrogen compounds and three types of sulfur compounds were considered as activators for addition polymerization of methacrylate monomers at mouth temperature. One of the major weaknesses of the self-curing resin filling materials is the high coefficient of thermal expansion. The loading of these resins may reduce the thermal expansion, but not to the range of tooth structure. Lack of color stability in the filling resins is another major discrepancy. Color stability can be improved by suitable modification of composition of the self-curing resin system. A method for measurement of color stability using the ASTM standard procedure with an electronic meter is described.

3. Dentistry and Mephenesin in Cerebral Palsy. *Manuel M. Album, Philadelphia Society for Crippled Children and Adults, Philadelphia, Pa.* Published in Proceedings of the 31st General Meeting of the I. A. D. R., *J. D. Res.* **32**: 634, 1953.

4. The Blood Supply of the Periodontal Ligament of the Rat. *Sylvio Bevilacqua, University of Rio de Janeiro, Brazil, and Gerard J. Keller, University of Pennsylvania, Philadelphia, Pa.*

*Charles A. Wilde, Jr., presiding.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH.

Scientific Proceedings of the British Section.
Guy's Hospital Dental School, London
March 21, 1953

Compiled by A. E. W. Miles, Editor, British Section

Index of Participants and Sequence Numerals for Corresponding Abstracts

*Armstrong, P. A., 11; Bradford, E. W., 9; *Crabb, H. S. M., 5; Darling, A. I., 5, 6, 7; Glasstone, S., 3; *Hay, M. F., 1, 2; Hodson, J. J., 15; Jenkins, G. N., 10, 11; *Little, K., 12, 16; *Lyon, D. G., 6; Miles, A. E. W., 8; *Pedler, J. A., 14; Rushton, M. A., 4; Stack, M. V., 13, 17; Wright, D. E., 10.

A London Section of the International Association for Dental Research was in existence for many years, but became inactive during the war. The great increase in the amount of dental research activity throughout the country has made the formation of a British Section expedient. The first meeting of the newly formed section was held at Guy's Hospital, London, on Saturday, March 21, 1953 and was attended by twenty members. Among the visitors were Lady May Mellanby, Sir Harold Himsworth, Secretary of the Medical Research Council, and Dr. T. F. Dixon, Secretary of the Bone and Tooth Society. Seventeen reports were presented by research workers from various parts of Great Britain. The British Section proposes to hold annual meetings in London, with additional meetings in other cities as the demand arises.

Much interest was aroused by the demonstration of appreciable quantities of material resembling enamel in mouse tooth germ cultures and by the growth of similar germs in chorio-allantoic membranes where thicker enamel was observed. A rare dentinal dysplasia related to hereditary opalescent dentin was discussed next, followed by an account of the application of grenz-ray studies to normal teeth and to three types of amelogenesis imperfecta. Polarized light was used in a study of the crystallike axes of enamel. Processes of the basal epithelial cells which appeared to contribute to the corium-epidermis bond in oral mucosa were demonstrated histologically. In dentin the translucent area surrounding the tubules was shown to be more highly calcified than the intertubular dentin matrix. Saliva had been examined with a view to correlating caries incidence with leukocyte count, and a number of antibacterial agents were detected in dog saliva. The relationship between collagen, reticulin, and mucopolysaccharide had been studied by electron microscopy and this was followed by a report on the carbohydrate hexosamine and peptide components of enamel. The only clinical study presented was on recurrent oral aphthae which were controlled by terramycin, but continued with reduced severity after treatment ceased.

1. Development of Mouse Tooth Germs in Tissue Culture. *Mary F. Hay, Dental School, University of Bristol.* Tooth germs from fetal mice have been grown in tissue culture in a way similar to that in which Glasstone (*J. Anat., London* **76**: 260, 1936; and *Proc. Roy. Soc., London* **126B**: 315, 1938) cultivated rat and rabbit tooth germs. The results on mouse tissue have

*Guests.

largely confirmed some of Glasstone's early work; the explants increased markedly in size, and cusps and apparently normal tubular dentin developed. In a small percentage of the mouse cultures an appreciable amount of an enamel-like substance also appeared. Glasstone does show a very thin layer of enamel in one of her rabbit culture photomicrographs. It also has been possible to show that in mice tooth germs are self-differentiating, at least up to the stage of dentin formation, from a very early age. A fragment of oral epithelium including part of the dental lamina rudiment at the two to three cell thick stage, plus a little underlying mesoderm, when explanted in vitro, developed in fourteen days into a quite well-shaped incisor with a fairly thick layer of dentin on the labial side. By excising other small fragments, whose position in the jaw is accurately known, it is hoped that the developmental potencies of various parts of the mammalian mandibular arch may be determined as Fell and Robison (*Quart. J. Microscop. Sc.* **82**: 563, 1941) have done for the chick.

2. The Fate of Meckel's Cartilage in the Mouse. *Mary F. Hay, Dental School, University of Bristol.* No satisfactory explanation of the fate of the central part of the mammalian Meckel's cartilage seems to have appeared in the literature. Various authors working on several different animals have described what happens at the symphysis of the jaw and also at the proximal end, but frequently that part of the cartilage between the two ends is said to merely disappear. It is also stated that phosphatase does not occur in Meckel's cartilage. This is not so in the mouse. Phosphatase has been demonstrated in Meckel's cartilage of a 17-day fetus and also of a newborn mouse. A process very similar to endochondral ossification seems to start in the cartilage about this time in the region of the lingual tip of the incisor root, and appears to progress anteriorly toward the symphysis and probably posteriorly toward the mandibular-squamosal articulation as well. Thus, for a time Meckel's cartilage is separated into an anterior and a posterior part. Bhaskar, Weinmann, and Schour (*J. D. Res.* **31**: 500, 1952) have recently recorded this separation in the rat, and have stated that endochondral ossification of the anterior part contributes to the forward growth of the mandible.

3. Further Progress in the Cultivation of Dental Tissues. *Shirley Glasstone, Department of Anatomy, University of Cambridge.* Simple tissue culture methods have serious limitations for the study of the development of dental tissues. The volume of tissue which can be maintained in health is small. The diffusion of nutrients is a slow process and limits the extent to which dentin and enamel are formed. The possibility of growing mammalian tissues in the highly vascular chorio-allantoic membrane of the chick or within the body cavity of the chick is therefore attractive. Tooth germs of both rat and mouse have been successfully grown in the chick chorio-allantoic membrane for ten days. Layers of dentin and a considerably thicker layer of enamel than previously seen in in vitro culture have been obtained. This is no doubt associated with the vascularization of the dentin papilla by the host capillaries, which is proved by the presence of nucleated red cells in the capillaries. The chorio-allantoic membrane is very rich in collagen and work is proceeding to determine whether this environment has any influence on the scleroproteins of the dentin of the graft.

4. Shell Teeth: A New Form of Dentinal Dysplasia. *Martin A. Rushton, Department of Dental Medicine, Guy's Hospital, London.* The teeth of a man of 21 years had enormous pulp cavities with thin dentin walls, the enamel being normal. The crowns were of normal shape, but the roots were short.

This shortness was not due to resorption, but in the upper jaw resorption had also taken place, and probably fracture of some roots. The abnormalities were less marked in the anterior teeth. Physical examination, chemical tests of the blood, and radiographs of the bones revealed no other abnormality. No relevant family history was found. Sections of two upper teeth showed them to be mere shells. Under the normal enamel was a thin layer of well-formed dentin ending in a zone where many tubules were dilated. On the pulp side of this was a still thinner layer of irregular calcified matrix. There was no evidence that any resorption had ever occurred. The pulp in one of the teeth was vital and presented an inactive appearance with coarse collagen bundles. The relationship of this dysplasia to hereditary opalescent dentin was discussed.

5. X-Ray Investigations of Human Dental Enamel. *H. S. Malcolm Crabb and A. I. Darling, Dental School, University of Bristol.* The characteristic radiation of various metals, chiefly copper, has been used to examine thin ground sections of normal human dental enamel for faults in calcification and of teeth with early carious lesions. Studies by Thewlis, Applebaum, Hollander, Van Huysen, and others have shown that Schreger's bands, enamel lamellae, early caries, and mottled enamel are associated with differences in density of calcification. The present study of clinically normal teeth has confirmed some of these findings and has shown further that in some teeth areas of decreased radiopacity were found over the cusps and close to the amelodentinal junction. These seem to correspond to recognizable histologic appearances. Areas of decreased radiopacity have also been shown in the enamel at the base of cracks or lamellae close to the amelodentinal junction. Histologically, they do not appear to be associated with caries, although they have been found to be related to enamel tufts. Further conclusions cannot be drawn at present. Applebaum's findings in early carious enamel have so far been confirmed and decalcification has been demonstrated in very early carious lesions.

6. Examination of Human Dental Enamel by Polarized Light. *D. G. Lyon and A. I. Darling, Dental School, University of Bristol.* This investigation was undertaken in the hope that it could be used in conjunction with the radiographic investigations of normal and abnormal enamel. So far an attempt has been made to investigate the crystallite axes of clinically normal human dental enamel following the work of Thewlis and others. The general findings of Thewlis have been confirmed and it has been shown that the mean deviation of the fiber axis of the crystallites in relation to the prism length in a maxillary lateral incisor and a maxillary premolar varied considerably from the amelodentinal junction to the enamel surface. The highest of about 200 readings for each tooth was found near the amelodentinal junction and the enamel surface. The deviation was consistently toward the cervical margin as described by Thewlis, and varied from 0 degrees to 44 degrees. Over the cusps the deviation decreased from either side and was mostly in the region of 0 degrees, deviating sometimes slightly away from the cervical margin. It is thought that such readings are due to the decussation of the prisms. An attempt to relate the varying deviations throughout the prisms to some morphologic feature, e.g., the striae of Retzius, has not yet been successful, although it is thought that some such relationship may exist. The suggestions of Thewlis and Keil that the 40 degree crystallites are predominantly related to the interprismatic substance does not seem to be borne out by these investigations.

7. X-Ray Investigation of Enamel in Amelogenesis Imperfecta. *A. I. Darling, Dental School, University of Bristol.* Cases diagnosed as hereditary and idiopathic types of amelogenesis imperfecta, in the accepted clinical sense of the term, have been studied. In each case all the teeth examined were affected. Macroscopically they fall into one or other of the two accepted groups, hypoplasia and enamel hypocalcification. Radiographic examination of sections revealed faults in calcification in all, including the hypoplasias. One case of hereditary hypoplasia showed a band of hypocalcification throughout the enamel, of one-third to one-half of its thickness, and lying close to the amelodentinal junction with the outer margin roughly parallel to the enamel surface. A group of cases showed a band of hypocalcification at the enamel surface and extending inward through about two-thirds of the enamel thickness. In one case all teeth had soft enamel on the occlusal third of the buccal surfaces. Radiographs, including those of unerupted teeth, showed major areas of hypocalcification at these sites. Minor faults were found in other parts of the enamel. Comparison of photomicrographs and radiographs showed that in no cases were enamel faults related to striae of Retzius, nor could the faults be related to the process of maturation as described by Diamond and Weinmann.

8. The Nature of the Attachment Between Surface Epithelial Cells and Corium of Human Oral Mucosa. *A. E. W. Miles, The London Hospital Dental School, London.* In skin cytoplasmic processes (Haftfasern or Wurzelfasern of German authors) project from the basal surfaces of the basal epithelial cells into the corium. Similar fingerlike basal processes, which no doubt contribute to the bond between epithelium and corium, also may be demonstrated in oral mucosa. Tentative views concerning the presence of a basement membrane and the relationship between the epithelial processes and connective tissue fibers at the boundary were put forward.

9. Observations on the Structure of the "Translucent Area" in Ortho-Dentin. *Eric W. Bradford, Department of Preventive Dentistry, University of St. Andrews.* The "translucent area" is more refringent, harder, and has a higher refractive index than the intertubular dentin matrix. These properties are compatible with the area, being more highly calcified than the intertubular dentin matrix. The "translucent area" varies considerably in size and properties from one animal to another. It appears to be missing in *Eptesicus serotinus*, an insectivorous bat, but comprises 75 per cent of the dentin matrix in *Equus caballus*. In man, in areas around carious lesions where a certain amount of decalcification is occurring, the "translucent area" is less translucent than the intertubular dentin matrix.

Perhaps the most important problem concerning the "translucent area" is that of its apparent dissolution in human material during decalcification and sectioning. There is little true dissolution but, even after careful decalcification with buffered formic acid, shrinkage occurs during dehydration and clearing, which leaves the matrix of the "translucent area" investing the dentinal process of the odontoblast in the center of the dentinal tubule. In the Eptesicus, where the original degree of calcification of the tissue is less, the matrix of the "translucent area" remains in its in vivo position during decalcification, but the elements involved are too small for accurate interpretation. Decalcified sections of the dentin of *Equus caballus*, however, demonstrate clearly the staining properties and relationships of the matrix of the "translucent area." These relationships are identical with those seen in ground sections of human ortho-dentin (Bradford: *Brit. D. J.* **90**: 303, 1951).

10. The Leukocyte Count of Saliva Specimens From Caries-Active and Caries-Free Subjects. *D. E. Wright and G. Neil Jenkins, Department of Physiology, Medical School, Newcastle.* Specimens of saliva were obtained from eleven caries-free and sixteen caries-active subjects either as spontaneous samples, in which the salivary contents of the mouth were emptied into a vessel, or as wax-stimulated samples. The stimulated saliva was collected for single five-minute periods or for five successive two-minute periods. These collections were made both with and without preceding periods of two minutes' duration of tooth brushing and mouth rinsing with distilled water. After thorough mixing, all specimens were examined on a hemocytometer slide under the phase-contrast microscope. The leukocytes could be divided under two headings: (1) those in well-preserved condition, referred to as "intact" cells, and (2) those in varying stages of disintegration. The cells in both these groups were classed together as total leukocytes. Counts were made of intact and total cells, and in all cases the number of intact leukocytes was greater in the samples from caries-free subjects than in those from caries-active subjects, the ratio varying from 2:1 to 7:1, dependent upon the method of collection. The total number of leukocytes was similar in the two groups of subjects irrespective of the method of collection. A check on the parodontal condition of our subjects was obtained in terms of the P.M.A. index of Massler, Schour and Chopra *J. Periodont.* **21**: 146, 1950 and a comparison showed that there was no difference in the state of gingivitis between the caries-free and the caries-active groups. Relative differences in inflammatory state of the gums do not therefore explain our observations. It is not yet known whether this higher percentage of intact cells in caries-free saliva is caused by increased numbers of cells entering the saliva or by a slower rate of disintegration, and the relation to any reduced bacterial activity in the caries-free mouth is also a problematical quantity.

11. Studies on the Antibacterial Substances in Dog Saliva. *Phyllis A. Armstrong and G. Neil Jenkins, Department of Physiology, Medical School, Newcastle.* By means of a questionnaire sent to veterinary surgeons and a survey of the literature, we have established that dogs are almost immune to caries. Antibacterial activity to eleven test organisms (among them bacteria which have been described as causing caries) has been demonstrated in every sample of saliva from more than 100 dogs kept under varying conditions. Incubation of the saliva for twenty-four hours increases its activity against three organisms, while incubation for forty-eight hours increases it against seven organisms and destroys the activity against two others. The incubated and nonincubated antibacterial factors have different properties on filtration as regards dialysis, treatment at various temperatures, exposure to ultra-violet light, and adsorption on charcoal, but both appear to be protein or protein derivatives. Lysozyme, a polypeptide, occurs in dog saliva in amounts having an average activity of 9 mg. per cent, with a range of from 0.1 to 32.5 mg. per cent of crystalline lysozyme (activity 10^6 units per gram) when assayed turbidimetrically using *Micrococcus lysodeikticus* as test organism. This amount of lysozyme is similar to that present in human saliva and did not account for the whole antibacterial activity of dog saliva against lysozyme-sensitive organisms and could not, in any case, account for activity against those which are lysozyme-insensitive. Evidence therefore exists for a multiplicity of antibacterial agents. Organisms which are potential antibiotic producers have been shown to be present in dog saliva as a result of "double pour plate" and streak technics using the eleven test organisms as before.

12. The Organic Constituents of Teeth. *K. Little, Atomic Energy Research Establishment, Berkshire.* Organic constituents of the teeth are being investigated using electron microscopy and x-ray diffraction. To identify components, teased out fragments from decalcified teeth, and in a few instances teeth from dermoid tumors, have been examined. So far, fragments have not been located more closely than as coming from enamel or dentin. At the outset it was known that considerable quantities of collagen occur in dentin, while keratin and reticulin had been suggested as components of enamel. Chemical analysis had also shown chondroitin sulfate. To verify these suggestions it was necessary to compare the fragments obtained with specimens of known material. The first problem was to determine the nature of reticulin: Samples have been obtained from a variety of tissues (Little and Kramer: *Nature, Lond.* **170**: 499, 1952) which were characterized histologically as reticulin, and in every case the appearance was of a membrane of uniform density containing a network of collagen fibrils. Indications were that the membrane contained a polysaccharide. Appearances of specimens obtained from dentin show that, in addition to collagen fibers, it contains reticulin. Membranes of keratin obtained from solutions of wool resembled, both in microscopic appearance and x-ray diffraction patterns, membranes from tooth enamel. So far, therefore, results indicate that enamel contains keratin; and dentin, collagen, and reticulin, the latter being a mixture of collagen and a polysaccharide. The possibility of other organic components is not excluded.

13. Nonprotein Organic Constituents of Enamel. *Maurice V. Stack, Department of Dental Medicine, Guy's Hospital, London.* Soluble and insoluble protein fractions have been prepared from enamel (*Brit. D. J.* **92**: 261, 1952), both in yields of 0.17 to 0.18 per cent, by demineralization with phosphoric acid. No hexose component other than glucose appeared to be present in these fractions, which were examined by heating with an orcinol-sulphuric acid mixture. Hexosamine was determined by acetylation and coupling with p-dimethylaminobenzaldehyde in acid solution. Values are recorded for the soluble protein fraction (glucose 9 per cent, hexosamine 2 per cent) and for the insoluble protein fraction (glucose 3 per cent, hexosamine 0.4 per cent). These together account for the amounts determined directly on the original enamel (glucose 0.02 per cent, hexosamine 0.004 per cent). There is evidence that the soluble protein is a glycoprotein. Carbohydrate and hexosamine could not be detected in the fraction from demineralized enamel which diffused through cellophane. Heating this fraction with a buffered ninhydrin reagent (*Oral Surg.* **4**: 1451, 1951) revealed that about one quarter of the nitrogenous material of enamel was probably in butanol-soluble peptide form. The amount of colored ninhydrin complex formed could be increased fivefold by preliminary hydrolysis of the diffusible fraction. About one-tenth of the peptide material demonstrated by this test could be recovered in aqueous extracts of enamel. No evidence of inhomogeneity was obtained by determining the ratio of ninhydrin-positive material to organic content in fractions collected from columns of powdered enamel (99.8 to 99.9 per cent pure). There was a direct relationship between the latter values when determined directly on eight 10-gram batches of enamel. Citric acid (0.10 per cent) is the only other nonprotein organic constituent of enamel known to have been determined (*J. D. Res.* **29**: 498, 1950).

14. The Effect of Terramycin Lozenges on the Incidence of Habitual Oral Aphthae: A Preliminary Report Based on Observations of Eleven Cases. *John A. Pedler, The London Hospital Dental School, London.* The

series consisted of five men and six women with histories of habitual oral aphthae of long duration. Some patients treated have been observed for periods up to two years. The lozenges used contain 15 mg. of terramycin and treatment has been varied in duration and daily dosage. The response in the majority of cases has been a period of freedom from ulceration. Two men who had suffered from oral aphthae practically without remission for periods of twenty and twenty-five years, respectively, were kept free of any lesions for three months by continuous administration of five or fewer lozenges daily. Ulcers then reappeared but were less frequent, less painful, and did not last so long. They also tended to occur singly rather than in crops. In the remaining cases, treatment in general has been restricted to several repeated courses of two to three weeks' duration only. It has been found that ulcers become painless and heal rapidly during these courses and fresh ulcers rarely develop. Although no patients have been cured, the condition has been considerably modified by topical terramycin treatment. The histology of the lesions in both the vesicular and ulcerated stages has been the subject of study.

15. Matrix Changes in the Caries Lesion. *J. J. Hodson, Department of Oral Pathology, University of Sheffield.* The caries lesion in normal, hypoplastic, and hypocalcified enamel has been studied in decalcified sections by microdissection of decalcified enamel and by the examination of ground sections using soft x-rays and other methods. Findings in decalcified and undecalcified material have been closely correlated. In very early white opaque carious lesions the demineralizing agent has little or no observable effect on the organic matrix. Later changes show increased chromophilia and apparent consolidation of the rods. Such matrix can safely be assumed to have been subjected to a demineralizing agent but not vice versa. The white opaque lesion shows no bacterial invasion and is usually acid-soluble. In pigmented lesions the matrix in bulk is brown, and in some cases a brown pigmented matrix can be recovered in sections. The pigmented lesion is acid-insoluble and shows denser consolidation, and may be confused with immature matrix. Bacterial invasion and apparent lysis are commonly found in this type of matrix, and are confined almost exclusively to the area of matrix consolidation. In immature matrix the effect of the carious demineralizing agent is variable. In some cases little or no effect is seen; in others there is heavy consolidation. There is a tendency to early bacterial invasion and lysis. Matrix consolidation appears to be due to penetration of organic material from the environment, and a change in the character of the enamel protein. The degree of consolidation is generally in direct relation to the proximity of the bacterial focus, but there is no apparent relation between it and the degree of demineralization.

16. Organic High Polymers in Teeth. *K. Little, Atomic Energy Research Establishment, Berkshire.* The greater proportion of the organic constituents of teeth are high polymers (collagen, keratin, and polysaccharide having been identified), and they must be expected to show those properties which are characteristic of high polymers. In particular, their molecular arrangement is partly ordered and partly disordered, with a variable degree of ordering. The lengths of the molecule are of the order of 500 Å, and the average size of the ordered regions is 50 to 80 Å in keratin, and perhaps several times that length in collagen, so that one molecule can pass through several ordered regions. Different intermolecular forces in ordered and disordered regions can result in different physical and chemical properties. Con-

sequently there will be sample-to-sample differences in those properties which are dependent upon degree of ordering. These include dye uptake, swelling and solubility, and rate of reaction (including both chemical degradation and solution). A further possible cause of variation is the random distribution of component units in the polymer molecules. Thus, in proteins there will be a tendency for glycine and alanine to occur in ordered regions and tyrosine in the disordered regions, while the proportion of components may vary from molecule to molecule. With such long molecules their length is rarely uniform, and the possibility arises that those molecular chains which are too short to pass through several ordered regions may be leached out in a swelling agent in which the polymer as a whole is not soluble, giving the superficial appearance of a separate chemical entity.

17. The Independence of Caries Experience and Salivary Tryptophane Content. *Maurice V. Stack, Department of Dental Medicine, Guy's Hospital, London.* Saliva was obtained under standard conditions and clarified before analysis. Difficulty was experienced in obtaining quantitative data with the reagents used by Turner and Crowell (*J. D. Res.* **26**: 99, 1947) for their qualitative study, from which they deduced that the presence of salivary tryptophane was correlated with resistance to caries. Satisfactory results were obtained by reacting saliva samples with p-dimethylaminobenzaldehyde in acid solution and adding a trace of nitrite. This method is similar to that used by Berg, Burrill, and Fosdick (*J. D. Res.* **25**: 231, 1946), but for their purposes the saliva was incubated under aerobic conditions for an hour before analysis. The mean, calculated from their pooled values, was 22.4 ± 9 (S.D.) micrograms per milliliter (34 cases grouped according to the periodontal condition). The mean salivary tryptophane content was 23 ± 8 (S.D.) micrograms per milliliter for the three subsidiary groups selected for the present investigation, and 22 ± 9 (S.D.) micrograms per milliliter for the main group (55 cases). When ranked in 5 μg steps, from 6 to 40 μg, the central values for each group were about half as numerous as would be expected for a normal distribution. Combining all groups (145 values), the numbers falling within these seven successive intervals were 5, 19, 39, 23, 38, 13, and 8, respectively; this anomalous distribution was not observed in a study of variation in salivary protein concentration by Gorlin (*J. D. Res.* **27**: 603, 1948). His results, together with those of previous workers, lead to the value 2.9 ± 0.6 (S.D.) micrograms per milliliter (186 cases). The correlation coefficient for DMF counts and salivary tryptophane levels was 0.10. It is considered likely that tryptophane was not observed previously in the saliva from caries-active patients because of the more rapid drop in redox potential of such saliva, as had been noted by the same authors.

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